HIV-1 INFECTION AMONG PREGNANT WOMEN IN ANGOLA: MOLECULAR EPIDEMIOLOGY AND TEST OF NOVEL AZT TRIAZOLE DERIVATIVES

CRUZ DOS SANTOS SEBASTIÃO

Tese para obtenção do grau de Doutor em Biomedicina

Doutoramento em associação entre:

Universidade NOVA de Lisboa (Faculdade de Ciências Médicas | NOVA Medical School)

Universidade de Aveiro

Julho, 2021

HIV-1 INFECTION AMONG PREGNANT WOMEN IN ANGOLA: MOLECULAR EPIDEMIOLOGY AND TEST OF NOVEL AZT TRIAZOLE DERIVATIVES

Cruz dos Santos Sebastião

Orientadores:

Rui Miguel Duque de Brito (EST&SL/IPL)

Joana Filipa Machado de Morais Afonso (INIS)

Helena Isabel Martins Soares (NMS|FCM/UNL)

Tese para obtenção do grau de Doutor em Biomedicina

Doutoramento em associação entre:

Universidade NOVA de Lisboa (Faculdade de Ciências Médicas | NOVA Medical School)

Universidade de Aveiro

Julho, 2021

INFEÇÃO PELO VIH-1 ENTRE MULHERES GRÁVIDAS EM ANGOLA: EPIDEMIOLOGIA MOLECULAR E TESTE DE NOVOS DERIVADOS TRIAZOLES DO AZT

Cruz dos Santos Sebastião

Orientadores:

Rui Miguel Duque de Brito (EST&SL/IPL)

Joana Filipa Machado de Morais Afonso (INIS)

Helena Isabel Martins Soares (NMS|FCM/UNL)

Tese para obtenção do grau de Doutor em Biomedicina

Doutoramento em associação entre:

Universidade NOVA de Lisboa (Faculdade de Ciências Médicas | NOVA Medical School)

Universidade de Aveiro

Julho, 2021

Para todas as pessoas infetadas com VIH que participam de forma livre nos estudos de investigação científica permitindo aos cientistas adquirirem conhecimento para melhorar o combate da pandemia do VIH/SIDA.

"*A tarefa não é tanto ver aquilo que ninguém viu, mas pensar o que ninguém ainda pensou sobre aquilo que todo o mundo vê*."

Arthur Schopenhauer (1788 – 1860)

ÍNDICE

Páginas

LISTA DE ABREVIAÇÕES

3TC — Lamivudina

ADN — Ácido desoxirribonucleico

ARN — Ácido ribonucleico

ARV — Antirretroviral

AZT — Zidovudina

CDC — Centro de Controle e Prevenção de Doenças

CRF — Formas Recombinantes Circulantes

dNTPs — Desoxirribonucleotídeos fosfatados

DST — Doenças Sexualmente Transmissíveis

EFV — Efavirenze

ETV — Etravirina

EUA — Estados Unidos da América

FTC – EmtricitabinaEntricitabina

IF — Inibidores de Fusão

IIN — Inibidores de integrase

IP — Inibidores de protéase

ITR — Inibidores de transcrição reversa

ITRN — Inibidores de transcrição reversa análoga de nucleosídeo/nucleotídeo

ITRNN — Inibidores de transcriptase reversa não análogo de nucleosídeo/nucleotídeo

ITS — Infeções de Transmissão Sexual

NVP — Nevirapina

OMS — Organização Mundial da Saúde

RDC — República Democrática do Congo

RPV — Rilpivirina

SIDA — Síndrome da Imunodeficiência Adquirida

SIV — Vírus da Imunodeficiência Símia

TARV — Tratamento antirretroviral

URF — Formas Recombinantes Únicas

VIH — Vírus da imunodeficiência humana

LISTA DAS FIGURAS

Páginas

LISTA DAS TABELAS

Páginas

LISTA DE PUBLICAÇÕES CIENTÍFICAS

I. **Cruz S. Sebastião**, Zoraima Neto, Domingos Jandondo, Marinela Mirandela, Joana Morais, and Miguel Brito. **HIV, hepatitis B virus, hepatitis C virus, and syphilis among pregnant women attending antenatal care in Luanda, Angola: seroprevalence and risk factors**. J Med Virol. 2020 Jun 9. doi: 10.1002/jmv.26148. Epub ahead of print. PMID: 32515502. https://onlinelibrary.wiley.com/doi/10.1002/jmv.26148

II. **Cruz S. Sebastião**, Zoraima Neto, Domingos Jandondo, Marinela Mirandela, Joana Morais & Miguel Brito. **Dengue virus among HIV-infected pregnant women attending antenatal care in Luanda, Angola: An emerging public health concern**.

III. **Cruz S. Sebastião**, Zoraima Neto, Carlos S. de Jesus, Marinela Mirandela, Domingos Jandondo, José C. Couto-Fernandez, Amilcar Tanuri, Joana Morais, and Miguel Brito. **Genetic diversity and drug resistance of HIV-1 among infected pregnant women newly diagnosed in Luanda, Angola**. PLoS One. 2019 Nov 26;14(11):e0225251. doi: 10.1371/journal.pone.0225251. PMID: 31770425; PMCID: PMC6879122. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0225251

IV. **Cruz S. Sebastião**, Joana Morais, and Miguel Brito. **Clinical and public health implications of HIV-1 genetic diversity and drug resistance mutations in Angola: a systematic review**. AIDS Rev. 2020 Oct 26;23(1):48-56. doi: 10.24875/AIDSRev.20000057. PMID: 33105474. https://www.aidsreviews.com/resumen.php?id=1547&indice=2021231&u=unp

V. **Cruz S. Sebastião**, Joana Morais, and Miguel Brito. **Factors influencing the HIV drug resistance among pregnant women in Luanda, Angola: Findings from a cross-sectional study**. Trop Med Infect Dis. 2021 Mar 5;6(1):29. doi: 10.3390/tropicalmed6010029. PMID: 33807796; PMCID: PMC8005960. https://www.mdpi.com/2414-6366/6/1/29

VI. **Cruz S. Sebastião,** *et al*. **Antiretroviral activity of novel 3'-Azidothymidine triazole derivatives against wild-type HIV-1** ***in vitro***.

AGRADECIMENTOS

A execução deste projeto de investigação biomédica não seria possível sem a criação de parcerias com instituições e investigadores africanos, americanos e Europeus que trabalham no campo das doenças infeciosas. As colaborações foram criadas graças ao elevado envolvimento e comprometimento da equipa de investigação biomédica composta pelos meus orientadores, o Professor Doutor Miguel Brito, a Professora Doutora Joana Afonso e a Professora Doutora Helena Soares que ao longo dos últimos quatro anos (2017–2020) criaram as condições necessárias para a execução do projeto. Por esta razão, gostaria de expressar a minha gratidão aos meus orientadores por todas as orientações, rigor científico e motivação.

Professor Doutor Miguel Brito, o meu orientador principal, com a sua inteira dedicação em todas as etapas do projeto, nomeadamente as etapas de iniciação, execução, monitoramento e encerramento, assim como a sua disponibilidade, paciência, foco e elevado senso de humor, foram cruciais para o sucesso do projeto. Além disso, a sua capacidade de gestão e liderança de projetos em saúde fez-me crescer como profissional na área de ciências biomédicas.

Professora Doutora Joana Afonso, a minha coorientadora, durante este período foi uma inspiração para a realização de pesquisa em virologia médica com elevado impacto nos programas de saúde pública. O seu entusiasmo em investigação biomédica, conhecimentos técnicos combinados com a sua capacidade de liderança de projetos em saúde, cooperação nacional e internacional, foram contribuições sem precedentes para o sucesso do projeto e para o meu crescimento profissional.

Professora Doutora Helena Soares, a minha coorientadora, as suas habilidades em pesquisa fundamental com elevado rigor na qualidade e análise dos dados foram cruciais para o sucesso do projeto e para o meu crescimento profissional. Eu não poderia imaginar um grupo de investigação fundamental melhor para a realização deste projeto com todos os seus aspetos desafiadores inerentes a uma pesquisa fundamental. Além disso, admiro as suas capacidades de liderança de equipas de investigação biomédica sobretudo no campo de imunologia e patologia humana, ao qual espero poder contar em futuras colaborações.

Outros investigadores e as suas respetivas instituições para o qual estou grato pelo suporte logístico, técnico, científico ou institucional são: Zoraima Neto, Domingos Jandondo e Marinela Mirandela do laboratório de biologia molecular do Instituto Nacional de Investigação em Saúde (INIS), pelo

suporte logístico, técnico e científico. Cristovão Domingos e Bárbara Pocongo do laboratório de biologia molecular do Instituto Nacional de Luta contra a SIDA (INLS), pelo suporte logístico. José Carlos Couto-Fernandez e Carlos Silva de Jesus do laboratório de virologia molecular da Fundação Oswaldo Cruz (FIOCRUZ), pelo suporte logístico, científico e treino em genotipagem do VIH-1. Amilcar Tanuri do laboratório de virologia molecular da Universidade Federal do Rio de Janeiro (UFRJ), pelo suporte científico. Maria José, Helena Salvador, Zabaca Pedro e Maria Oliveira da Maternidade Lucrécia Paim (MLP), pelo suporte logístico e clínico. Joaquim Castigo Levita, Moisés Dembo, Pedro Castelo, Maria Afonso, Idalina Luamba, Ludovina Bartolomeu e Ricardo Mucusa do Instituto Superior de Ciências da Saúde (ISCISA) da Universidade Agostinho Neto (UAN), pelo suporte logístico e institucional. Sofia Cerqueira, Juliana Gonçalves, Daniela Silva, Ana Tomé, Inês Monteiro e Rafael Tajuelo do laboratório de imunologia e patogénese do Centro de Estudos de Doenças Crónicas (CEDOC), pelo suporte logístico e técnico. Cláudia Andrade e Ana Oliveira do CEDOC pelo suporte logístico e técnico. Ana Petronilho e Daniel Alencar do Instituto de Tecnologia Química e Biológica António Xavier (ITQB), pela síntese e disponibilização generosa dos novos compostos derivados do AZT. Valter Nuaila e Márcio Sitoe da Universidade Eduardo Mondlane (UEM), Fredilson Melo do ITQB e Sarah Hill da University of Oxford, pelo suporte científico. Agradecimentos ao INIS, Centro de Investigação em Saúde de Angola (CISA), INLS, MLP, FIOCRUZ, ISCISA/UAN, CEDOC, Centro de Investigação em Saúde e Tecnologia (H&TRC), Escola Superior de Tecnologia e Saúde de Lisboa (EST&SL), Instituto Politécnico de Lisboa (IPL) e Instituto Português do Sangue e da Transplantação (IPST), pelo suporte institucional. Ao projeto Pró-África (CNPq: 440145/2015–5) por suportar algumas análises. Ao Instituto Gulbenkian de Ciência (IGC), Programa de Pós-Graduação Ciência para o Desenvolvimento (PGCD), Fundação Calouste Gulbenkian e a Fundação para a Ciência e a Tecnologia (FCT), pela atribuição da bolsa de doutoramento (Concessão número SFRH/BD/135296/2017).

Finalmente, gostaria de expressar a minha gratidão a todas as mulheres grávidas que participaram deste estudo de investigação biomédica, a minha querida família, aos meus colegas, amigos e em particular a minha esposa (Joana Mavila Kinanga Sebastião) e o meu filho (Daniel Kinanga Sebastião), pelo companheirismo e compreensão que a minha ausência durante estes últimos quatro anos foi necessário para a execução e conclusão deste projeto.

Muito obrigado a todos que direita ou indiretamente contribuíram para o sucesso do projeto.

CONSIDERAÇÕES GERAIS

Os resultados apresentados nesta tese de doutoramento refletem o trabalho realizado durante os últimos quatro anos (2017–2020) no campo de doenças infeciosas, nomeadamente o estudo dos aspetos relacionados com a disseminação do VIH em mulheres grávidas de Luanda, a cidade capital de Angola, com principal ênfase na epidemiologia molecular do VIH-1 e o desenvolvimento de uma nova geração de compostos derivados do AZT com eficiência para inibir o ciclo de replicação do VIH-1. O meu interesse em pesquisa de doenças infeciosas começou durante a minha licenciatura em Análises Clínicas e Saúde Pública no ISCISA/UAN onde tive a oportunidade de trabalhar como estagiário no rastreio hematológico, serológico e parasitológico de doenças infeciosas no hospital Josina Machel (HJM) e no Instituto Nacional de Sangue (INS), em Luanda. Os desafios intelectuais enfrentados durante este período, levou-me a desenvolver o interesse de pesquisa no campo de doenças infeciosas, sobretudo nos países tropicais e subtropicais que além do VIH são também endémicos com outras doenças infeciosas causadas por agentes etiológicos virais ou bacterianos.

Uma vez ingressado no Programa de Pós-Graduação Ciência para o Desenvolvimento (PGCD) (http://pages.igc.gulbenkian.pt/pgcd/pt/about/pgcd) e posteriormente ingressado no programa de doutoramento em biomedicina da NOVA Medical School (NMS), Faculdade de Ciências Médicas (FCM) da universidade NOVA de Lisboa (UNL), em conjunto com a Universidade de Aveiro, adquire habilidades técnicas e científicas eficazes para a aplicação em projetos de investigação de alto nível no campo de ciências médicas. Sob orientação do Professor Doutor Miguel Brito, Professora Doutora Joana Afonso e a Professora Doutora Helena Soares, tive a oportunidade de desenvolver habilidades técnicas e científicas robustas em biologia molecular, genotipagem do VIH-1, bioinformática, imunologia, patologia, saúde pública e escrita científica. Além disso, tive a oportunidade de participar em projetos multinacionais focados em investigação epidemiológica de doenças infeciosas virais com uma equipa de investigadores altamente motivados do Instituto Nacional de Investigação em Saúde de Angola (INIS) e da Universidade de Oxford.

Como doutor em biomedicina, o meu interesse primário de pesquisa inclui a investigação da epidemiologia molecular de doenças infeciosas, os seus fatores de risco e a implementação de intervenções eficazes impactantes no controlo de doenças infeciosas e melhoria da qualidade de saúde pública. Estes interesses de pesquisa surgiram do facto de a investigação de agentes infeciosas, assim como os determinantes comportamentais ou sociodemográficos que influenciam

o surgimento e a disseminação de doenças infeciosas ser crucial para a eficácia de qualquer programa de controlo de doenças infeciosas sobretudo nos países de recursos limitados. Além disso, investigar as doenças infeciosas em mulheres grávidas é um bom estimador epidemiológico da frequência de doenças infeciosas em crianças e que pode contribuir na redução da mortalidade materna, insucesso da gravidez e efeitos adversos nos recém-nascidos.

Devido à coerência e relevância dos manuscritos elaborados e publicados nos últimos quatro anos, foi decidido pela equipa de investigação do projeto, compilar todos os trabalhos científicos obtidos neste projeto e apresentar em alternativa a tese de doutoramento, de acordo com os regulamentos da NMS|FCM/UNL (Capítulo III, artigo 23.º, Diário da República, 2.ª série — N.º 111–9 de junho de 2015). Acreditamos que a apresentação do relatório final deste projeto na forma de trabalhos publicados em revistas com reconhecido mérito internacional e com comités de seleção e revisões por pares antes da publicação, permitirá maior acesso e divulgação dos resultados para a comunidade científica. Os conteúdos parciais destes trabalhos foram usados para a elaboração desta tese. Declaro que todos os manuscritos publicados e apresentados nesta tese, foram eticamente aprovados por comités nacionais e/ou institucionais e o consentimento foi obtido para cada participante, os seus familiares ou responsável legal depois de os objetivos dos estudos serem apresentados e explicados. Além disso, tive um papel importante no desenho dos estudos, colheita e análise dos dados, processamento laboratorial, escrita, edição e revisão crítica dos manuscritos, seleção das revistas e aprovação dos manuscritos para a publicação.

Esta tese está estruturada em cinco diferentes capítulos. No primeiro capítulo, apresentamos uma introdução geral sobre os principais marcos epidemiológicos da pandemia do VIH/SIDA. No segundo capítulo, apresentamos os objetivos do projeto. No terceiro capítulo, apresentamos todos os manuscritos gerados e/ou publicados durante o período de investigação. No quarto capítulo, fizemos uma análise e discussão geral dos principais resultados e as suas implicações clínicas para o manejo da pandemia do VIH/SIDA em Angola. No quinto e último capítulo, descrevemos as principais conclusões e recomendações dos futuros estudos que poderão vir a ser realizados além dos que foram apresentados nesta tese.

CAPÍTULO 1: Introdução

1.1 História da pandemia do VIH/SIDA

Entre os anos 1979 – 1981, casos incomuns de patologia severa causadas por *Pneumocystis pneumonia*, sarcoma de kaposi, linfoadenopatia generalizada e outras infeções oportunistas começaram a ser relatados em homens que fazem sexo com homens (HSH) que viviam nas regiões metropolitanas dos Estados Unidos da América (EUA)[1,2]. Naquela altura, essas complicações estavam limitadas a pessoas que apresentavam imunossupressão, o que despertou a atenção do centro de controle e prevenção de doenças (CDC) dos EUA, sobre a possibilidade da disseminação de um novo agente infecioso causador de imunossupressão[3].

Inicialmente, os casos estavam restritos a HSH, o que sugeriu que o novo agente infecioso poderia ser transmitido pelo contacto sexual[4]. Posteriormente, foi descrito quadro clínico semelhante em utilizadores de drogas injetáveis, indivíduos que receberam transfusão de sangue, parceiros sexuais de pessoas imunodeprimidos e crianças nascidas de pais imunodeprimidos[5]. Assim sendo, em setembro de 1982, o CDC declarou a emergência de uma nova doença infeciosa com múltiplos modos de transmissão causadora de imunodeficiência, o que levou a doença a ser denominada de síndrome da imunodeficiência adquirida (SIDA)[6]. Desde então, cientistas de todo o mundo uniram esforços e começaram a investigar o agente etiológico, a sua origem, os modos de transmissão, mecanismos de infecciosidade e patogenicidade. Em maio de 1983, um retrovírus linfotrópico que mostrou associação com a SIDA foi isolado e denominado vírus da imunodeficiência humana (VIH)[7].

1.2 Origem da pandemia do VIH/SIDA

A descoberta do VIH como sendo o agente etiológico da SIDA foi uma das principais conquistas científica verificada durante o século XX[8]. Após o isolamento e caracterização do VIH em 1983, o vírus da imunodeficiência símia (SIV) foi isolado de diferentes espécies de primatas tais como, macacos, gorilas e chimpanzés presentes nas regiões central e ocidental de África que apresentavam deficiências imunológicas similares ao causado pelo VIH[9–11]. A estreita relação filogenética observada entre as sequências do SIV nestes primatas, mostrou que os primatas africanos eram os reservatórios naturais do SIV, e que o vírus poderia ter atravessado a barreira das espécies, infetado os humanos, replicado e diversificado em inúmeras variantes (Figura 1)[12].

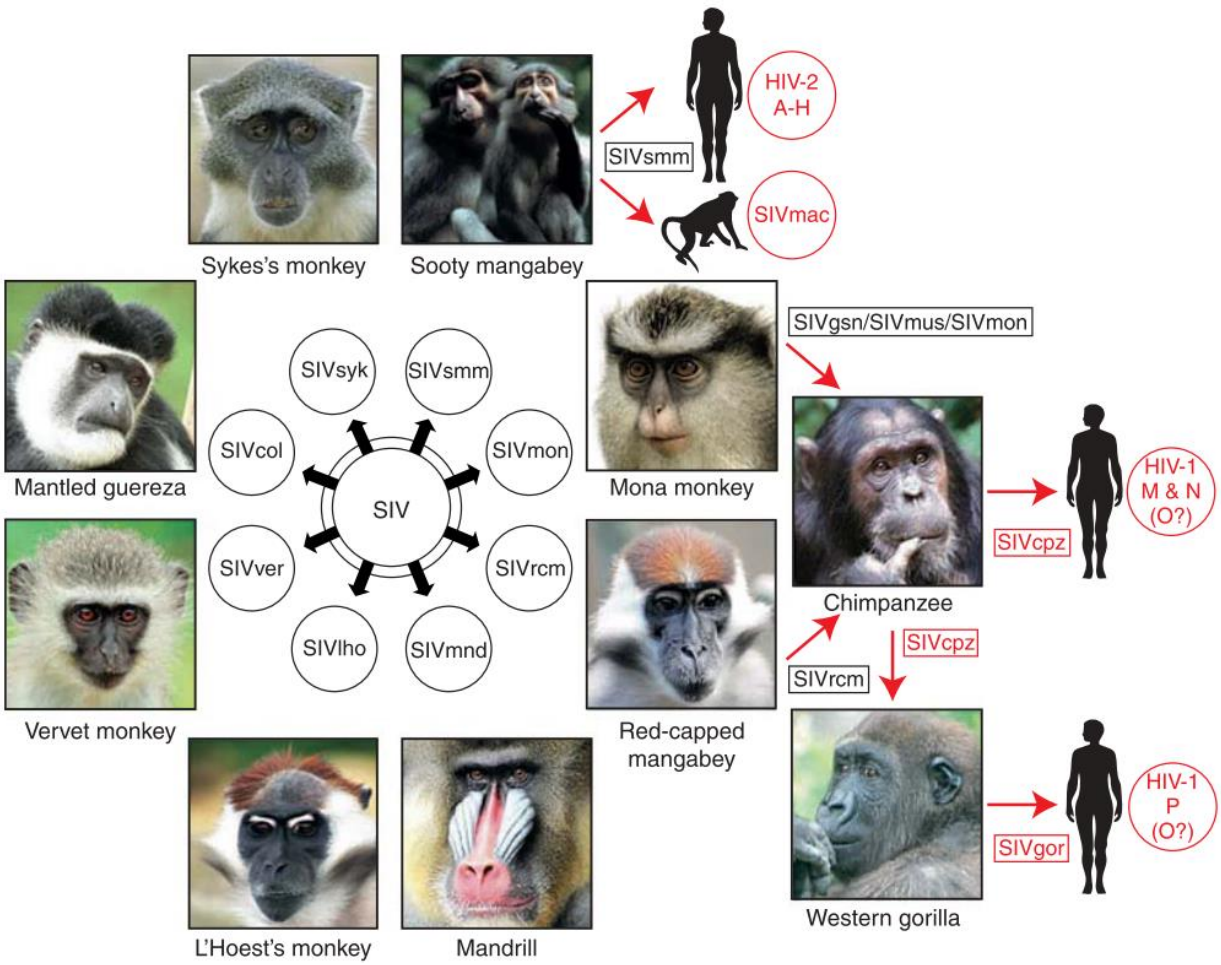


Figura 1 — Origem da pandemia do VIH/SIDA. Os primatas são naturalmente infetados com diferentes lentivírus chamados SIV. Vários desses vírus cruzaram a barreira das espécies infetando humanos e diversificando em novas categorias de agentes infeciosos virais. (Copiado da referência 12)

Curiosamente, apesar do SIV ser conhecido como um vírus capaz de induzir imunodeficiência similar ao causado pelo VIH, poucas alterações imunológicas têm sido observada nos seus reservatórios naturais mesmo na presença de elevado nível de replicação viral, o que sugere que o processo de introdução e evolução do SIV em primatas não humanos africanos poderia ter ocorrido durante um longo período de tempo[13]. Assim sendo, a elucidação dos mecanismos pelos quais os hospedeiros naturais do SIV evitam a progressão da doença devem ser mais explorados, de modo a fornecer mais informações da patogénese do VIH que podem levar à identificação de novos alvos terapêuticos para reforçar o combate da pandemia do VIH/SIDA[9–11]. Por outro lado, evidências moleculares mais antigas mostraram que existe uma estreita relação filogenética do SIV com o VIH, o que sugeriu que a pandemia do VIH/SIDA poderia ter originado de transmissão zoonótica de primatas não humanos para os humanos ocorridos em regiões de África[12,14].

Nos últimos anos, estudos moleculares e filogeográficos têm feito a reconstrução da origem e difusão do VIH numa escala espacial e temporal[15]. Estes estudos estimaram que a pandemia do VIH/SIDA teve início por volta dos anos 1920 na atual República Democrática do Congo (RDC), localizado na região central de África[16]. Além disso, os mesmos estudos estimaram que o paciente zero que poderia ter tido várias transmissões individuais que posteriormente procederam à transmissão homem – homem e começado a pandemia do VIH/SIDA, adquiriu o SIV entre os anos 1900 – 1930 em algum lugar na região central de África, nomeadamente na RDC, Camarões, Gabão, Guine equatorial, Congo Brazzaville, República Centro Africana ou Angola[17].

Mesmo que não tenha nenhum impacto no curso da pandemia do VIH/SIDA, compreender os fatores que permitiram a transmissão bem-sucedido do SIV de primatas não humanos para os humanos é importante como uma obrigação moral para com as vítimas do VIH/SIDA, mas também para permitir tirar lições importantes que podem ajudar a humanidade a evitar enfrentar pandemias semelhantes no futuro[18]. Assim sendo, os fatores que levaram à ocorrência desta transmissão zoonótica bem sucedida de primatas não humanos para os humanos, continua a ser um mistério, apesar de estudos sugerirem que provavelmente a caça e o comércio de carne de animais selvagens, mordidas causadas por primatas mantidos como animais de estimação, exposição a secreções e sangue durante a manipulação da carne, ou carcaça do animal para ser cozinhado por um caçador, ou a sua esposa, serem apontadas como as possíveis vias de transmissão do SIV de primatas não humanos para os humanos (Figura 2)[14].

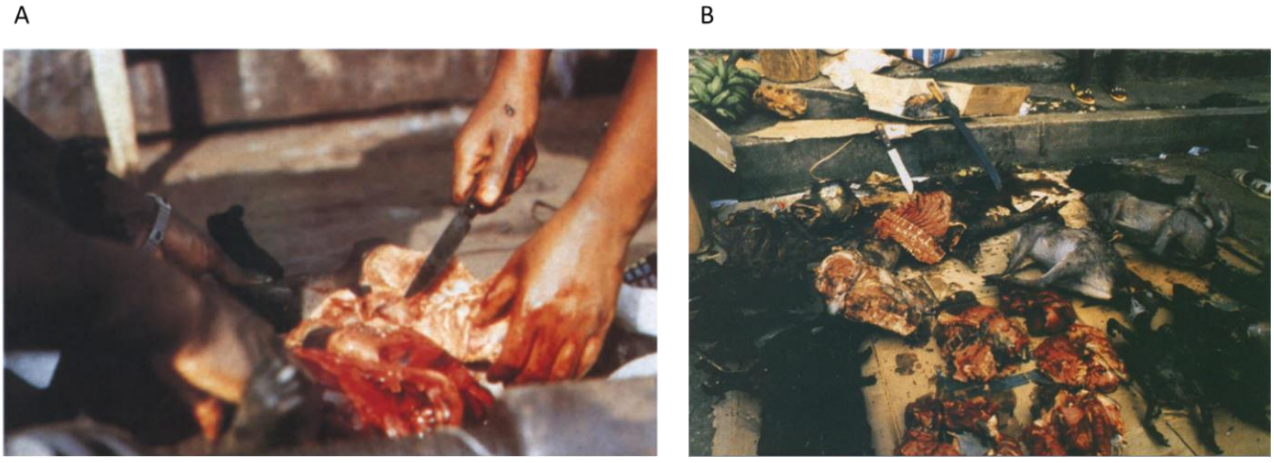


Figura 2 — VIH/SIDA como uma zoonose. (A) Exposição humana ao sangue de primata durante a preparação de alimentos. (B) Mercado de carne de caça no centro-oeste da África. (Copiado da referência 14)

1.3 Epidemiologia do VIH/SIDA

O VIH representa um dos agentes infeciosos com mais impacto para a saúde pública[19]. Embora a infeção tenha afetado todos os países, algumas regiões são mais afetadas, principalmente o continente africano que representa mais de 50% das infeções e em alguns países desta região, mais de um em cada cinco indivíduos adultos podem apresentar a infeção (Figura 3)[20].

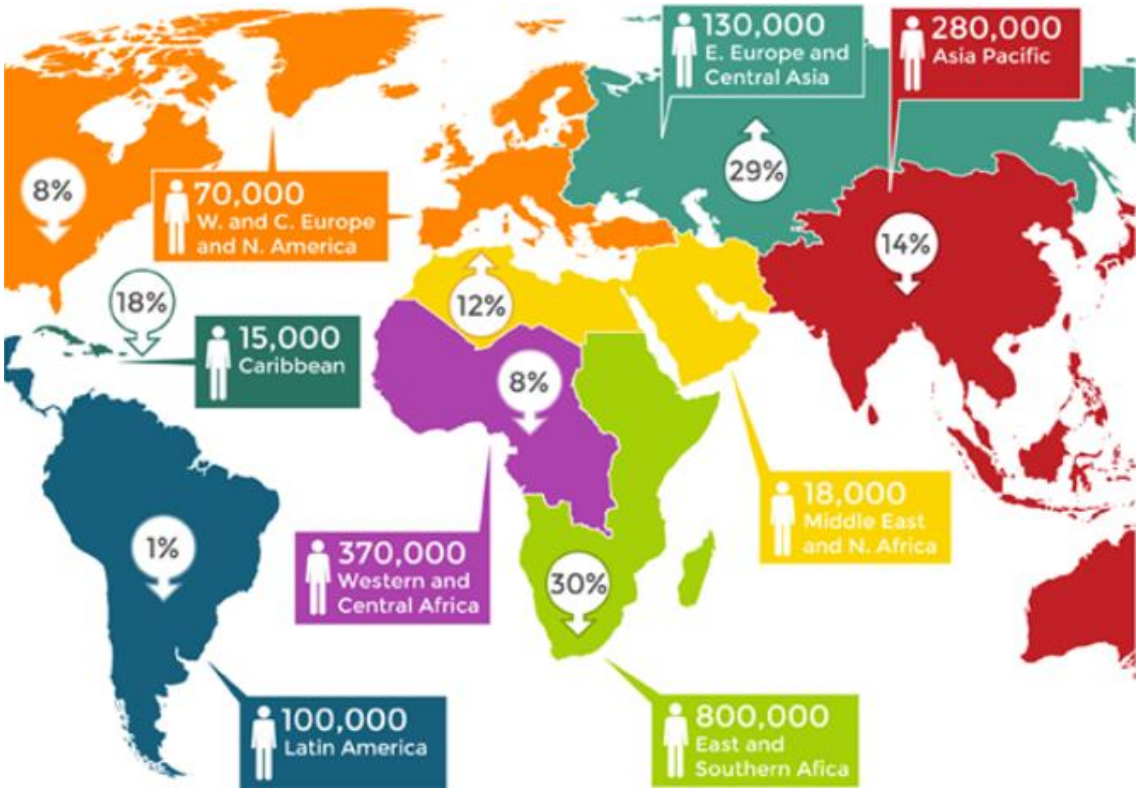


Figura 3 — Distribuição epidemiológica dos casos ativos do VIH. AVERT (Adaptado de https://www.avert.org/global-hiv-and-aids-statistics. (Acedido aos 21 de dezembro de 2020)

Quatro décadas após o aparecimento dos primeiros casos do VIH, o balanço epidemiológico global de incidência e mortalidade continua trágico[19]. A epidemia continua a se espalhar rapidamente e o número de pessoas portadoras do VIH subiu de 8 milhões em 1990 para cerca de 38 milhões em 2020, com mais de 750 000 mortes relacionados com a infeção em todo o mundo, desde que surgiram os primeiros casos entre 1979 – 1981[20]. Estima-se que 70% de todas as infeções e 50% de todas as mortes relacionadas com o VIH/SIDA são de pessoas residentes em África[19]. Devido a sua escala e impacto devastador, a epidemia tem sido caracterizada como uma emergência mundial e um dos maiores desafios, afetando o desenvolvimento socioeconómico, individual, familiar e comunitário [21,22]. Além disso, o VIH/SIDA causa repercussões profundas na expectativa de vida dos indivíduos infetados bem como no crescimento económico dos países[23].

Em África, as infeções de transmissão sexual (ITS) são responsáveis por elevadas taxas de mortalidade e morbilidade com elevado impacto na saúde da população, sobretudo na população jovem[24]. A característica epidemiológica da epidemia causada pelo VIH difere de região para região em todo o mundo e depende de fatores de risco biológico e riscos comportamentais que afetam o ritmo de crescimento da epidemia e a capacidade de resposta dos países para combater a infeção[25]. Por isso, estudos locais capazes de identificar estratégias para reduzir o número de novas infeções e mitigar o sofrimento imposto aos indivíduos, a família e a sociedade, devem ser implementados principalmente nos países em desenvolvimento que apresentam maiores índices de infeção[26]. Em África, mais de 80% das infeções pelo VIH são adquiridas de forma heterossexual, enquanto a transmissão vertical e a transfusão de sangue contaminado são responsáveis pelas infeções restantes[27]. Na América Latina, a maioria das infeções pelo VIH são adquiridas por HSH e pelo uso indevido de drogas injetáveis, por outro lado, o contato heterossexual e a injeção de drogas são os principais modos de transmissão do VIH na Asia[27].

O risco de transmissão vertical do VIH em gestantes seropositivas sem qualquer intervenção, é entre 15–45% e a transmissão ocorre principalmente durante o período intraparto[28]. A falta de diagnóstico precoce de doenças infeciosas durante a gravidez e o aleitamento materno também têm sido apontados como fatores cruciais de exposição e transmissão materno-infantil do VIH, pois uma mulher grávida seropositiva pode passar o vírus no seu bebé durante a gravidez, durante o parto ou durante a amamentação, porque o vírus está presente no leite materno e pode ser transmitido para a criança durante a amamentação[20]. O início precoce da atividade sexual, condições socioeconómicas precárias, fluxo migratório em parte devido aos conflitos políticos ou guerras, desemprego, baixo nível de escolaridade e comportamentos sexuais de risco, têm sido fatores sociodemográficos associados com a transmissão do VIH em mulheres jovens com idades abaixo dos 25 anos, sobretudo em países de baixa e média renda[29].

1.3.1 Epidemiologia molecular do VIH-1 e 2

A infeção causada pelo VIH é mundialmente caracterizada pela presença de heterogeneidade genética considerável no seu genoma, sendo agrupados em VIH-1 e VIH-2. O VIH-1 é o responsável por causar infeções, a nível mundial, enquanto o VIH-2 tem estado restrito sobretudo em alguns países da região da África ocidental, central e alguns casos na Europa e América[12]. A infeção causada pelo VIH-2 é caracterizado por progressão lenta da doença, o paciente apresenta elevado nível de

linfócitos T CD4 e carga viral baixa, enquanto o VIH-1 é caracterizado por progressão rápida, carga viral elevada, replicação viral persistente e depressão imunológica[30,31]. O VIH-1 compreende quatro grupos diferentes, o grupo M (*Main*), N (*non-M*, *non-O*), O (*outlier*) e P[32]. Até onde sabemos, apenas três espécies de primatas (chimpanzés, gorilas e mangabe fuliginosos) transmitiram os seus vírus aos humanos, o SIVcpz do chimpanzé deu origem aos grupos M e N no homem e foi também transmitido ao gorila dando origem ao SIVgor que depois deu origem aos grupos O e P no homem (Figura 1)[18]. Atualmente, o grupo M é o responsável pela pandemia do VIH/SIDA, enquanto os grupos N, O e P têm sido reportados em baixas prevalências (menos de 2%) principalmente nas regiões ocidental e central de África[33]. Além disso, o grupo M ainda é subdividido em dez subtipos (A-D, F-H, J-L) e oito subsubtipos (A1-A6, F1 e F2) que foram particularmente relacionados aos subtipos A e F porque não têm constituição genética suficientemente distante para constituírem novos grupos ou subtipos do VIH-1[34,35]. O VIH-1 também conta com mais de 100 formas recombinantes circulantes (CRFs) e formas recombinantes únicas (URFs)[34]. As CRFs constituem formas híbridas resultantes de um evento de recombinação entre dois subtipos diferentes do VIH-1 desde que seja observado em três ou mais indivíduos que não apresentam vínculo epidemiológico, enquanto as URFs são variantes sem grupo filogenético definido observado em um ou mais indivíduos com vínculo epidemiológico[34].

Devido a elevada variabilidade genética do VIH, o uso de ferramentas de estudo molecular aplicado a epidemiologia, tais como a análise filogenética, tem sido usado para ajudar a caraterizar e compreender a transmissão e disseminação do vírus nas diferentes regiões e populações[15]. O uso destas ferramentas, ajuda a compreender melhor as formas de transmissão e permite que as autoridades sanitárias definam estratégias para reduzir as taxas de infeção, principalmente nos grupos de elevado risco[26]. A região central de África é a que apresenta maior diversidade genética do grupo M, sendo considerado como o epicentro da pandemia do VIH/SIDA, onde possivelmente teria ocorrido a transmissão zoonótica[14]. Por outro lado, a distribuição mais heterogénea nos outros continentes, é um forte indicativo do efeito fundador, ou seja, que houve introdução e disseminação de algumas variantes do VIH-1 que se tornaram predominantes nestas regiões. Os subtipos C, A e CRF02_AG são predominantes no continente africano, enquanto o subtipo B predomina no continente Europeu e Americano e a CRF01_AE predomina no continente asiático (Figura 4)[36]. Segundo estimativas da distribuição molecular dos subtipos do VIH-1, o subtipo C contribui com

mais infeções, à escala global, representando cerca de 46,6%, seguido dos subtipos B (12,1%), A (10,3%), G (4,6%), D (2,7%), F, H, J e K agrupados com 0,9%[36].

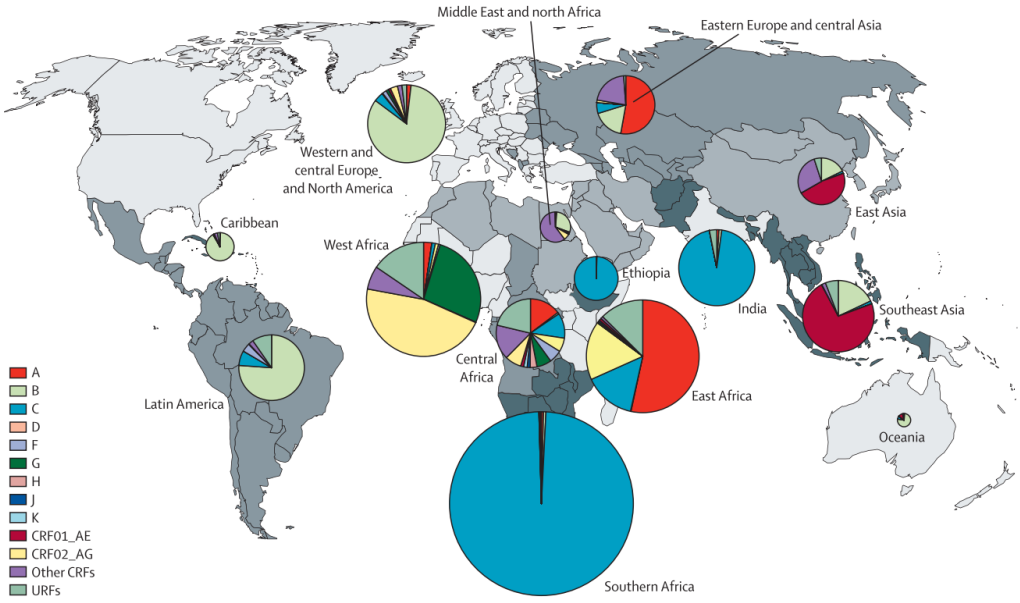


Figura 4 — Distribuição geográfica dos subtipos do VIH-1. As proporções de subtipos de HIV-1, CRFs e URFs diferiu entre as regiões. A diversidade genética é maior na África Central, onde todos e muitos subtipos recombinantes são encontrados. (Copiado da referência 36)

1.4 Estrutura e mecanismo de patogenicidade do VIH-1

O VIH-1 pertence ao género dos *Lentivírus* da família *Retroviridae* caracterizados por causarem imunossupressão, ativação imunológica inapropriada, replicação viral persistente e longo período de latência clínica[37]. Estruturalmente, o VIH-1 é uma estrutura esférica microscópica composta por um genoma de duas moléculas idênticas do ácido ribonucleico (ARN) que codifica as proteínas do invólucro viral (glicoproteínas gp41 e gp120), proteínas estruturais (matriz, capsídeo e nucleocapsíde), e as enzimas transcriptase reversa, integrase e protéase (Figura 5)[38].

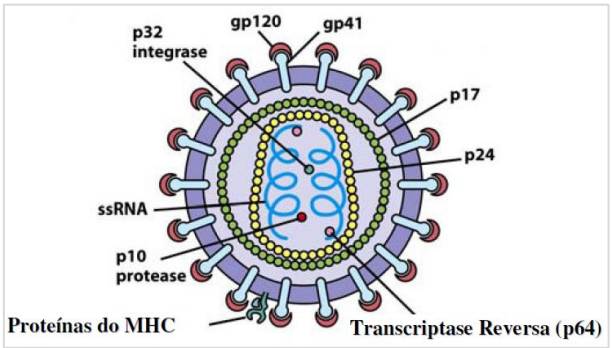


Figura 5 – Estrutura do VIH. Externamente, o VIH é constituído por uma bicamada lipídica que é derivada da célula do hospedeiro e as glicoproteínas gp120 e gp4. Internamente, o VIH é constituído por uma matriz proteica (p17) que envolve o capsídeo viral (p24) onde se encontra o genoma viral associadas a três enzimas virais, denominada transcriptase reversa, integrasse e protéase. (Copiado da referência 38)

O ciclo de replicação do VIH-1 compreende os estágios de ligação, entrada na célula, libertação do ARN viral, transcrição reversa, integração do ácido desoxirribonucleico (ADN) proviral no genoma do hospedeiro, síntese proteica e libertação de novas partículas virais (Figura 6)[39].

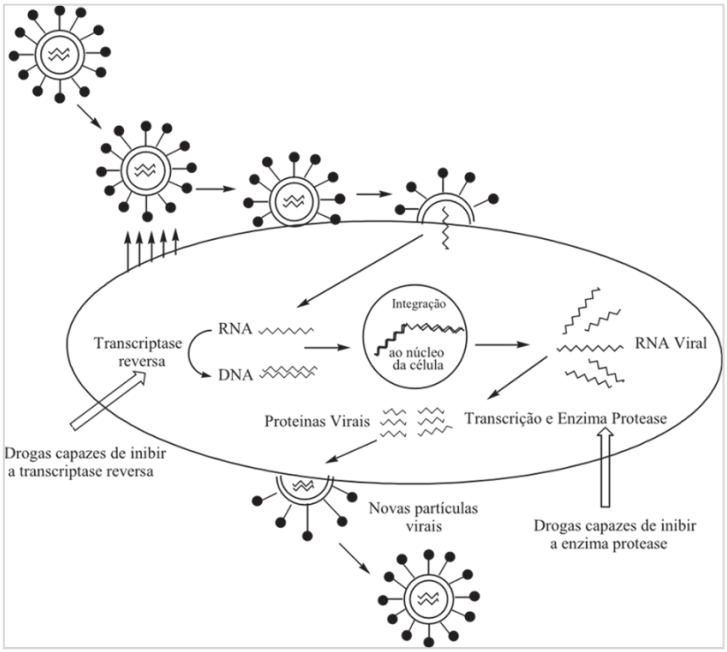


Figura 6 — Esquema geral do ciclo de replicação do VIH-1. Inicialmente, o VIH se funde com a membrana da célula do hospedeiro e penetra na célula. Após a entrada, o VIH libera o seu material genético para ser convertido em DNA. Após a conversão, o DNA viral funde-se com o DNA da célula. Finalmente, o DNA viral é transcrito e traduzido em partículas virais capazes de infetar novas células. (Copiado da referência 39)

Após entrada no organismo, o VIH liga-se na superfície dos linfócitos, macrófagos, eosinófilos, células dendríticas e microgliais[40]. Normalmente, o VIH começa por infetar os linfócitos T expressando o coreceptor CCR5[41]. As variantes do VIH que utilizam as células com coreceptores CCR5 são conhecidos como vírus R5, enquanto as variantes do VIH que utilizam os coreceptores CXCR4 são conhecidos como vírus X4[42]. Além disso, existem variantes com duplo tropismo ou vírus X4R5, que apresentam a capacidade de ligação nos dois coreceptores (CCR5 ou CXCR4)[43,44]. Após a ligação do vírus com o recetor CD4 e os coreceptores CCR5 ou CXCR4, as membranas do vírus fundem-se com as membranas celulares ocasionando a entrada de partículas virais no interior da célula hospedeira[45,46]. Uma vez na célula, o vírus liberta as moléculas de ARN que se ligam a enzima transcriptase reversa para dar o início da síntese do ADN complementar que posteriormente é integrado na forma de ADN proviral no genoma da célula hospedeira através da ação da enzima integrase[47]. Um estudo recente sobre tecnologias de célula única aplicadas à pesquisa do VIH-1, revelou que a frequência de células VIH-1 positivas portadoras de um único provírus integrado é 85–90%, sugerindo que apenas uma minoria de células infetadas pode sustentar a recombinação,

um mecanismo importante para a evolução viral[48]. Finalmente, o ARN mensageiro resultante da transcrição do ADN proviral migra para o citoplasma resultando na produção de proteínas estruturais, funcionais e acessórias, tornando a nova partícula viral madura e infecciosa[49–51].

A figura 7 resume as etapas de progressão natural do VIH/SIDA e a dinâmica de variação da carga viral e as células T CD4. Após a contaminação o paciente entra na fase aguda da doença caracterizada por apresentar elevação da carga viral com quadro clínico assintomático. Durante este período, a resposta imune adaptativa pode manter parcialmente a infeção e a replicação viral controlada. Posteriormente, o paciente entra na fase crónica da doença que pode durar meses ou mesmo anos. Nesta fase, o paciente apresenta latência clínica, infeção dos órgãos linfoides, redução da carga viral e equilíbrio na taxa de destruição e produção de linfócitos T CD4. Com a progressão da doença para SIDA, haverá um declínio gradual no número de linfócitos T CD4, e dessa forma, o sistema imunitário do paciente fica enfraquecido, tornando o paciente vulnerável a contrair infeções oportunistas. Nesta fase o paciente pode apresentar sintomas característicos da infeção pelo VIH/SIDA como o aparecimento de febre, cefaleia, linfoadenopatia, anorexia, artralgia, mialgia, vómito, diarreia e exantema[52]. O estádio clínico de monitorização da progressão da doença para SIDA é definido principalmente pela quantificação dos linfócitos T CD4 em estágio 1 (≥500 células/µL), estágio 2 (entre 200 e 499 células/µL) e estágio 3 (<200 células/µL)[53].

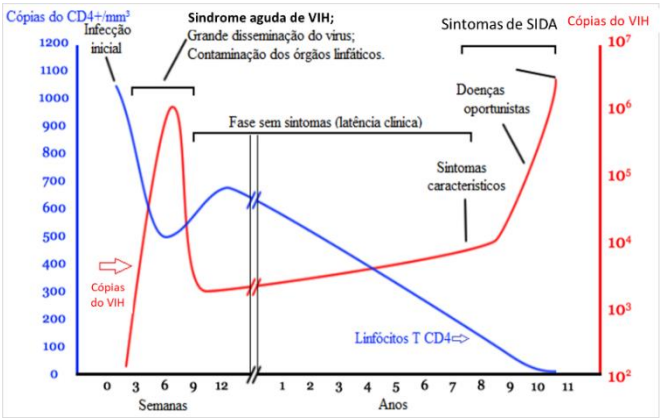


Figura 7 — Curso clínico da infeção pelo VIH-1. A fase aguda da doença inicia após a contaminação caracterizado por apresentar quadro clínico assintomático. Após meses ou mesmos anos, o paciente entra na fase crónica da doença, caracterizado principalmente pela redução da carga viral. Com a progressão da doença, haverá declínio de linfócitos T CD4, tornando o paciente vulnerável a contrair infeções oportunistas. (Adaptado de https://pt.wikipedia.org/wiki/Ficheiro:Hiv_timecourse_(pt).png; (Acedido aos 06.10.2020)

1.5 Tratamento da infeção do VIH-1

Desde o surgimento dos primeiros casos de infeção pelo VIH, uma variedade de abordagens terapêuticas têm sido aplicadas para combater a infeção, incluindo, a remoção de células

portadoras de vírus, transfusões de linfócitos, transplante do timo e de medula óssea, entretanto, o progresso mais significativo no desenvolvimento de estratégias terapêuticas contra o VIH/SIDA, tem sido o desenvolvimento de compostos antirretrovirais (ARVs)[54]. Atualmente, o uso de ARVs no combate da pandemia do VIH/SIDA é uma realidade mundial e os preços são cada vez mais acessíveis, o que faz com que os países com recursos limitados tenham acesso aos ARVs para garantir o controlo da epidemia localmente[55].

Inicialmente, o uso de ARVs dependia da contagem de linfócitos T CD4 ou através da carga viral, mas atualmente, as diretrizes de tratamento antirretroviral (TARV) recomendam o início do tratamento logo após a confirmação da infeção, independentemente do estadio clínico em que o paciente se encontra[56]. O principal objetivo do início do TARV é reduzir a virémia plasmática a níveis indetetáveis, reduzir a progressão e mortalidade da doença, resgatar e preservar o sistema imunológico do paciente[55]. A adesão ao TARV é um elemento fundamental capaz de garantir o sucesso terapêutico, impedir a ocorrência de doenças oportunistas, evolução dos parâmetros laboratoriais e seleção de estirpes com mutações capazes de conferir resistência aos ARVs e diminuir as opções terapêuticas[57]. Os alvos dos ARVs são proteínas envolvidas na entrada do vírus na célula do hospedeiro e as enzimas virais[55]. Atualmente, os ARVs aprovados pela agência americana de regulamentação dos medicamentos são classificados de acordo com a capacidade de interromper uma etapa específica do ciclo de replicação do VIH como: inibidores de fusão (IF), inibidores de transcrição reversa (ITR), inibidores de integrase (IIN) e inibidores de protéase (IP) (Tabela 1)[58].

Tabela 1 — Lista de ARVs aprovados pela agência americana de regulamentação dos medicamentos (Adaptado da referência 51)

ITR		**Inibidores de Protéase**	**Inibidores de Integrase**	**Inibidores de Fusão**
ITRN	**ITRNN**			
Zidovudina (AZT)	Efavirienz (EFV)	Amprenavir (APV)	Raltegravir (RAL)	Enfurvitida (T20)
Abacavir (ABC)	Nevirapina (NVP)	Atazanavir (ATV)	Dolutegravir (DTG)	
Didanosina (ddI)	Etravirina (ETV)	Darunavir (DRV)	Bictegravir	
Tenofovir (TAF)	Rilpivirina (RPV)	Indinavir (IDV)	Cabotegravir	
Lamivudina (3TC)	Doravirina	Lopinavir/r (LPV)		
Tenofovir (TDF)		Nelfinavir (NFV)		
Zalcitabina (ddC)		Fosamprenavir (FPV)		
Emtricitabina (FTC)		Ritonavir (RTV)		
		Saquinavir (SQV)		

Os IF são ARVs que interferem com o processo de fusão do envelope viral com a membrana da célula do hospedeiro[59,60]. Os ITR foram os primeiros ARVs a serem aprovado pela agência americana de regulamentação dos medicamentos para o tratamento de pacientes infetados pelo VIH[61,62]. Atualmente, esta classe de ARV esta dividida em análogos de nucleosídeo/nucleotídeo (ITRN) e não análogo de nucleosídeo/nucleotídeo (ITRNN)[63]. Os ITRN sofrem fosforilação de enzimas celulares e conseguem mimetizar de forma competitiva os contactos estruturais dos desoxirribonucleotídeos fosfatados (dNTPs) endógenos (adenosina, citosina, timidina e guanina) no local ativo da enzima TR do vírus[62,63]. Além disso, os ITRN perdem o 3'-hidroxil na ribose e com isso, após a incorporação durante o processo de transcrição reversa, obrigam a terminação do processo de transcrição reversa devido à interrupção da formação de pontes fosfodiésteres necessária para a síntese e estabilização do DNA proviral[64,65]. Por outro lado, os ARVs da classe dos ITRNN são inibidores não competitivos que induzem alteração conformacional da enzima transcriptase reversa que afeta a atividade catalítica da enzima, e dessa forma, impede a transcrição reversa do ARN em ADN.[66] Os IP inibem a atividade da enzima protéase impedindo a clivagem das proteínas virais e as suas subunidades funcionais, o que impede a formação de novas partículas infeciosas[67,68].

A descoberta dos inibidores de integrase capazes de impedir a incorporação do ADN proviral no genoma do hospedeiro, e dessa forma, não haverá a produção de partículas virais ou síntese de novos vírus, foi considerado um marco importante para o combate da pandemia do VIH/SIDA[69]. Os ARVs desta classe têm sido bem tolerados e eficazes, e por esta razão, têm sido fortemente recomendados para a sua inclusão como parte de todos os regimes de primeira linha de TARV, principalmente nos países de baixa e média renda[70].

O composto zidovudina (AZT) apresentando o grupo 3'-Azido que impede a formação de ligações fosfodiéster 5'-3' interrompendo o processo de alongamento da cadeia de ADN proviral, foi o primeiro composto da classe dos ITRN a ser aprovado em 1987, sob o nome comercial de Retrovir, pela agência americana de regulamentação dos medicamentos para o tratamento do VIH/SIDA[71]. Dois anos depois da aprovação do AZT, a agência americana de regulamentação dos medicamentos aprovou a didanisona (ddI), sob o nome comercial de Videx e em 1991 foi instituído a terapia dupla com AZT e ddI. Posteriormente em 1995, a agência americana de regulamentação dos medicamentos aprovou o saquinavir (SQV), um ARV da classe dos IP, reforçando o cenário de tratamento dos pacientes com VIH/SIDA. O uso combinado de ARV de diferentes classes contribui

significativamente na redução da mortalidade e incidência de doenças oportunistas em pacientes infetados com VIH[55].

Os esquemas de TARV de primeira linha deve consistir em dois ITRN mais um ITRNN ou um inibidor de integrase (Tabela 2)[55]. O esquema contendo TDF+3TC (ou FTC)+EFV como uma combinação de dose fixa é recomendado como a opção preferida para iniciar o TARV, entretanto, se este esquema estiver contraindicado ou não disponível, uma das seguintes opções alternativas é recomendada: AZT+3TC+EFV, AZT+3TC+NVP ou TDF+3TC (ou FTC)+NVP[55]. Os esquemas TDF+3TC (ou FTC)+DTG ou TDF+3TC (ou FTC)+EFV podem ser usados como opções alternativas para iniciar o TARV[55]. A TARV combinada mostrou ser bastante eficaz no controlo da infeção causada pelo VIH, entretanto, a interação entre os ARVs, a toxicidade e o surgimento de variantes com mutações de resistência tem sido fatores que contribuem para a busca de novos compostos com atividade ARV melhorada e com baixa ou nenhuma citotoxicidade[72].

Tabela 2 — Regimes de TARV de primeira linha para adultos, mulheres grávidas ou amamentando, adolescentes e crianças. (Adaptado da referência 65)

Primeira linha de TARV	Esquema preferencial	Esquemas alternativos
Adultos	TDF + 3TC (ou FTC) + EFV	AZT + 3TC + EFV (ou NVP) TDF + 3TC (ou FTC) + DTG TDF + 3TC (ou FTC) + EFV TDF + 3TC (ou FTC) + NVP
Mulheres grávidas ou amamentando	TDF + 3TC (ou FTC) + EFV	AZT + 3TC + EFV (ou NVP) TDF + 3TC (ou FTC) + NVP
adolescentes	TDF + 3TC (ou FTC) + EFV	AZT + 3TC + EFV (ou NVP) TDF (ou ABC) + 3TC (ou FTC) + DTG TDF (ou ABC) + 3TC (ou FTC) + EFV TDF (ou ABC) + 3TC (ou FTC) + NVP
Crianças de 3 a 10 anos	ABC + 3TC + EFV	ABC + 3TC + NVP AZT + 3TC + EFV (ou NVP) TDF + 3TC (ou FTC) + EFV (ou NVP)
Crianças menores de 3 anos	ABC (ou AZT) + 3TC + LPV/r	ABC (ou AZT) + 3TC + NVP

1.5.1 Descoberta de novos compostos antivirais

O processo de desenvolvimento e descoberta de novos compostos químicos com atividade antiviral contra o VIH-1 tem contribuído na redução da morbilidade e mortalidade associados a pandemia do VIH/SIDA nas últimas décadas. Atualmente, existem mais de 30 compostos químicos antivirais

utilizados para o tratamento do VIH/SIDA[55]. Entretanto, devido os numerosos efeitos colaterais, intolerância ou toxicidade decorrentes do uso prolongado destes compostos, obriga a comunidade científica a desenvolver constantemente novos compostos antivirais mais económicas, com aumento da sua eficiência e com pouco ou nenhum efeito colateral[73].

A enzima transcriptase reversa do VIH-1 é um alvo terapêutico com eficácia clínica comprovada cientificamente[74]. Mesmo assim, a busca por novos compostos com novos mecanismos de ação capazes de inibir a ação da transcriptase reversa do VIH-1, continua necessária e prometem reduzir ainda mais o risco do surgimento de variantes com resistência. Para tal, os novos compostos devem apresentar eficácia quanto a inibição da replicação viral, evitar eventos adversos que são típicos dos inibidores licenciados desta classe de ARVs e devem ser capazes de combater a infeção por estirpes resistentes que continua a ser uma das principais causas de falha do tratamento[75].

O AZT é um dos ARVs mais utilizados nos esquemas de TARV de primeira linha em muitos países em desenvolvimento[28]. Os efeitos colaterais mais comuns decorrentes do uso do AZT incluem dores de cabeça, febre e náuseas, que dificulta a adesão de muitos pacientes em tratamento com esquemas contendo o AZT[76]. Entretanto, estudos anteriores mostraram que uma modificação apropriada na estrutura química do AZT pode aumentar a eficácia antiviral do composto, adicionar novas funcionalidades e reduzir os efeitos colaterais[77–81]. A presença do grupo azida substituindo o 3'-OH da desoxirribose na estrutura química do AZT, converte este composto antiviral num substrato adequado para modificação e introdução de novos compostos orgânicos tais como compostos aromáticos ou hidrocarbonetos capazes de melhorar a atividade antiviral do composto[77–82].

Uma das técnicas mais utilizadas atualmente no processo de otimização e descoberta de novos compostos antivirais é a técnica de "química do click" que permite juntar substratos orgânicos com biomoléculas específicas[83,84]. A descoberta desta técnica melhorou drasticamente o processo de síntese e descoberta de compostos capazes de inibirem a replicação do VIH-1. Mesmo assim, a busca de outras abordagens terapêuticas que vão além da inibição da replicação viral pode se tornar o foco na descoberta de compostos antivirais contra o VIH-1 nas próximas décadas[85]. Além disso, estratégias para reforçar a resposta imunológica, combater a infeção nos reservatórios latentes ou usar terapia genética para proteger as células da infeção pelo VIH-1 também têm sido exploradas e promete ser um campo de muita atividade científica nas próximas décadas[86].

1.6 Resistência ao TARV

O entendimento da base genética capaz de conferir resistência aos ARVs é importante para ajudar a prevenir a disseminação de estirpes do VIH-1 com resistência aos antivirais numa determinada população. Os testes para identificação de resistência ao TARV baseiam-se em sequenciação do fragmento do genoma viral amplificado através da reação em cadeia da polimerase (PCR - Polimerase Chain Reaction), que posteriormente são sequenciados e comparado com uma sequência reconhecida como suscetível aos ARVs[87]. A realização de genotipagem do VIH-1, através do sequenciamento do gene *pol*, permite a identificação da população viral e a presença de mutações que conferem resistência aos ARVs contra as principais classes do ARVs, os ITR e IP, que são amplamente usados nos esquemas de TARV de primeira e segunda geração, principalmente nos países em desenvolvimento[87].

Globalmente, mais de 10% dos pacientes infetados pelo VIH-1 apresenta alguma mutação associada à redução da resposta ao TARV tanto em países de baixa, média ou alta renda[88]. Entre 2014 e 2019, a prevalência média de transmissão de resistência aos fármacos foi de 4% no Sul/Sudeste Asiático, 6% na África Subsaariana, 9% na América Latina/Caribe, 8,5% na Europa e 14% no Norte América[89]. Durante o mesmo período, houve aumento na resistência geral aos ITRN/ITRNN na África Subsaariana; aumento na resistência aos ITRNN na América Latina/Caribe e aumento em aos ITRNN e IP na América do Norte[89]. A elevada taxa de mutação durante a transcrição reversa (cerca de uma mutação para cada 2000 nucleotídeos incorporados pela enzima TR do vírus) tem sido associado a ausência da atividade de revisão exonucleotídica no sentido 3'→5' pela enzima TR do vírus[47]. Dessa forma, as mutações resultantes da pressão seletiva devido à resposta imunológica ou falha virológica devido a pouca adesão ao tratamento, tornam-se predominantes, resultando em novas variantes do VIH-1, *quasispecies* ou subtipos recombinantes com diferentes mecanismos de patogenicidade capazes de conferir a habilidade do vírus escapar da resposta imune e desenvolver resistência ao TARV[90]. A mutação M184V que confere resistência contra os ITRN e as mutações G190A, K103N e Y181C que conferem resistência contra os ITRNN são a mais frequentes nas mais diversas regiões de África (Figura 8)[91]. A mutação M184V reduz a suscetibilidade viral aos fármacos Lamivudina (3TC) e Emtricitabina (FTC), a mutação G190A reduz a suscetibilidade viral ao fármaco nevirapina (NVP), a mutação K103N reduz a suscetibilidade viral ao fármaco efavirenze (EFV) e NVP, e finalmente a mutação Y181C reduz a suscetibilidade viral aos fármacos NVP, etravirina (ETV) e rilpivirina (RPV)[92,93].

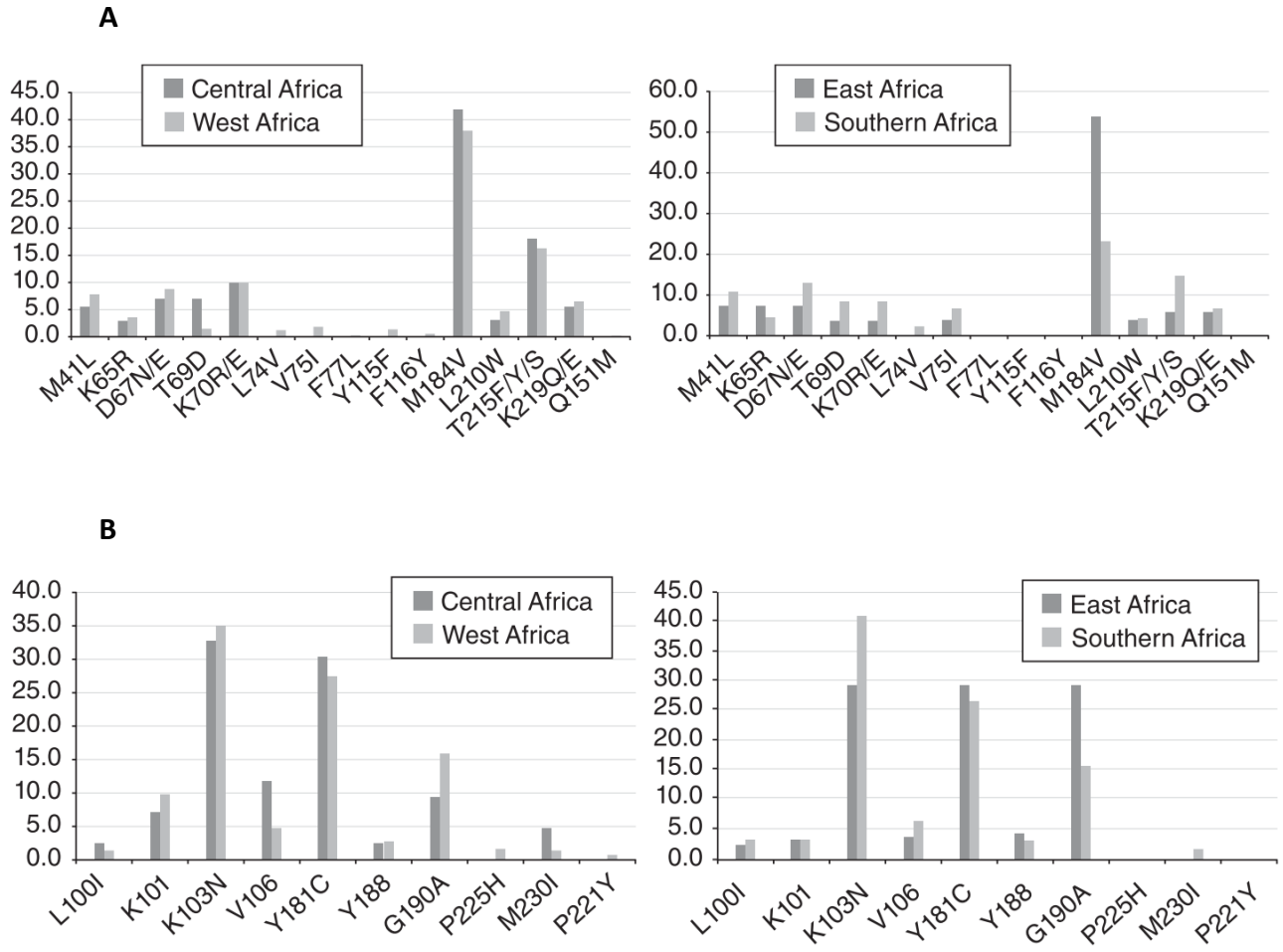


Figure 8 — Prevalência de mutações de resistência nos ARVs em África. (A) Mutação nos ITRN (B) Mutação nos ITRNN. A principal mutação do ITRN em todas as quatro regiões é a M184V. Na África Ocidental e Central, a principal mutação dos ITRNN é a K103N, na África Oriental é a K103N e Y181C, e na África do Sul também é a K103N. (Copiado da referência 91)

A frequente identificação de variantes do VIH-1 com mutações de resistência, sobretudo nos países de baixa e média renda, representa uma potencial ameaça para o sucesso a longo prazo da TARV e a eliminação da pandemia do VIH/SIDA como um problema de saúde pública global até 2030[94–96]. Assim sendo, a busca de novos compostos capazes de inibir a replicação de diferentes variantes do VIH-1 incluindo variantes com mutações de resistência, tem sido, sem dúvida o progresso mais importante para o combate da pandemia do VIH/SIDA[97], uma vez que o uso de ARV para tratar a infeção do VIH é crucial para a preservação do sistema imunológico do paciente, para reduzir a viremia, reduzir a transmissão viral, reduzir a progressão da doença para SIDA, melhorar a qualidade de vida dos pacientes e reduzir a mortalidade relacionada ao VIH/SIDA[98]. Por esta razão, investigadores de todo o mundo, têm investigado estratégias alternativas para a descoberta de compostos antivirais com diferentes modos de ação, incluindo o combate da infeção persistente

nos reservatórios virais para reforçar a disponibilização de mais opções de tratamento para o VIH/SIDA[85,86]. Atualmente, muitos estudos têm documentado resultados promissores de compostos com modificação química no grupo 3'-Azido do AZT, mostrando ser uma estratégia promissora para a descoberta de novos compostos com atividade antiviral[77–84].

Uma das metas da OMS para controlar o VIH/SIDA é a garantia de que nenhuma criança exposta ao VIH-1 durante o período gestacional apresenta a infeção, sobretudo variantes resistentes aos ARVs[99]. Para tal esforço global tem sido feito para desenvolver compostos antivirais baratos e eficientes no combate da infeção e infeção resistente aos ARVs, além disso, o acesso universal e precoce ao diagnóstico da infeção causada pelo VIH-1, pela triagem nos grupos de riscos, em mulheres de idade fértil ou mulheres grávidas durante as consultas pré-natal têm sido recomendadas[100]. Estas estratégias visam permitir o diagnóstico precoce da infeção, sobretudo em mulheres grávidas, para implementar o protocolo de transmissão vertical de modo a reduzir as hipóteses de transmissão da infeção do VIH em neonatos e para que as pessoas infetadas possam ter a garantia do exercício saudável ou seguro da reprodução, principalmente nos países em desenvolvimento[99].

1.7 Epidemiologia do VIH/SIDA em Angola

Angola é um país do sudoeste da África, dividida em 18 províncias com uma superfície de 1.246.700 km^2 que tem fronteira a norte com a República do Congo e RDC, a sul tem fronteira com a Namíbia, a este tem fronteira com a Zâmbia e a Oeste é banhada pelo Oceano atlântico. Durante um longo período, Angola manteve guerra colonial e guerra civil, o que levou a deslocação da população para o exterior do país, sobretudo para os países que fazem fronteira com Angola. No contexto pós-guerra, Angola tem enfrentado vários desafios para combater a pobreza, a reconstrução das infraestruturas económicas e sociais, reinserção social e o combate de epidemias causadas por agentes infeciosos, dentre os quais o VIH[101]. Estudos anteriores realizado na região da África Subsariana mostraram que existe relação quanto a propagação do VIH e as condições sociodemográficas da população[102]. Assim sendo, as dificuldades sociodemográficas enfrentadas pela população angolana durante e após os conflitos armados no país, fez com que surgissem muitos fatores de risco tais como o desemprego, pobreza extrema, baixo nível de escolaridade, início precoce da atividade sexual, aumento de trabalhador sexual, serviços de saúde e infraestruturas

debilitadas que favoreceram ao longo dos últimos anos a disseminação do VIH e outras doenças infeciosas nas diferentes regiões do país.

Os primeiros casos de infeção pelo VIH/SIDA em Angola, foram reportados em 1985 em Luanda, a cidade capital do país, e desde então, a infeção tem se espalhado rapidamente em baixa prevalência principalmente das regiões urbanizadas para as regiões não urbanizadas do país[101,103]. Até o final do ano 2020, 330 000 infeções e 14 000 mortes relacionadas com o VIH/SIDA foram reportados em Angola[20]. Destas, 38 000 infeções e 4,900 mortes foram registadas em crianças da faixa etária dos 0–14 anos, muitas delas resultantes de transmissão da infeção de mão para os seus filhos[20]. Atualmente, a prevalência média nacional do VIH/SIDA em Angola encontra-se abaixo dos 3% na população com idades entre os 15–49 anos, com maior prevalência nas mulheres em comparação aos homens com a mesma idade (Figura 9)[101].

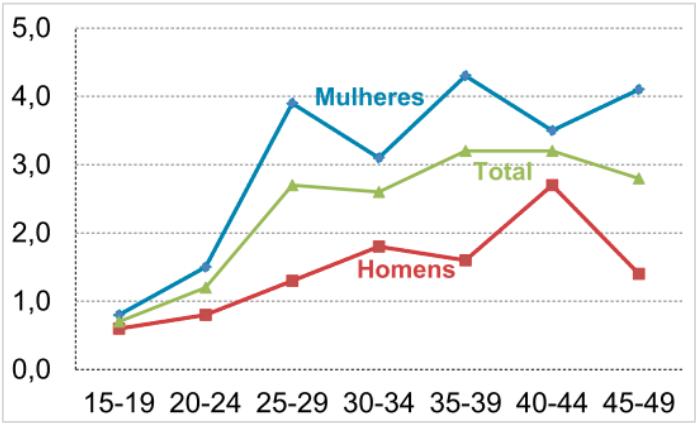


Figura 9 — Prevalência de VIH por sexo e idade em Angola. A prevalência do VIH em Angola é predominada por mulheres. A prevalência mais baixa é inferior a 1% nos adolescentes de 15 – 19 anos, tanto nos homens como nas mulheres. Entre as mulheres, o valor máximo verifica-se nas faixas etárias de 25 – 39 anos, enquanto nos homens, o valor máximo é observado na faixa de 40 – 44 anos. (Copiado da referência 101)

Os dados de prevalência do VIH/SIDA em Angola, têm sido estimados com base em estudos periódicos de seroprevalência incluindo gestantes em seguimento nas consultas pré-natal e outros grupos de risco de infeção e disseminação de doenças infeciosa[101]. Entretanto, devido à periodicidade irregular na realização destes estudos bem como o limitado número de participantes, estes estudos não representam toda a população infetada e o atual cenário de doenças infeciosas em Angola. A transmissão da infeção por VIH em Angola é predominada por meio das relações heterossexuais[103]. As províncias do norte do país registam as prevalências mais baixas (0,5% – 0,9%) da infeção pelo VIH, enquanto as províncias do sul e do leste registam as prevalências mais altas (4,0% – 6,1%)[101].

1.7.1 Epidemiologia molecular do VIH-1 em Angola

A informação sobre epidemiologia molecular do VIH/SIDA em Angola continua a ser escassa, apesar de alguns estudos revelarem a existência de grande diversidade genética dos subtipos do VIH-1 nas diversas regiões norte, centro e sul do país[33,104]. O padrão de distribuição molecular do VIH-1 em Angola é muito heterogéneo, com maior prevalência a ser observada para os subtipos C, F1 e CRF02_AG[105–113]. A pandemia do VIH/SIDA na região sul de Angola é dominada pelo subtipo C, na região norte é dominada pelo subtipo F1, enquanto na região central é dominado pelos subtipos C e F1, apesar de se observar uma grande frequência de variantes recombinantes, sobretudo a CRF02_AG e o recombinante U/H (Figura 10)[110].

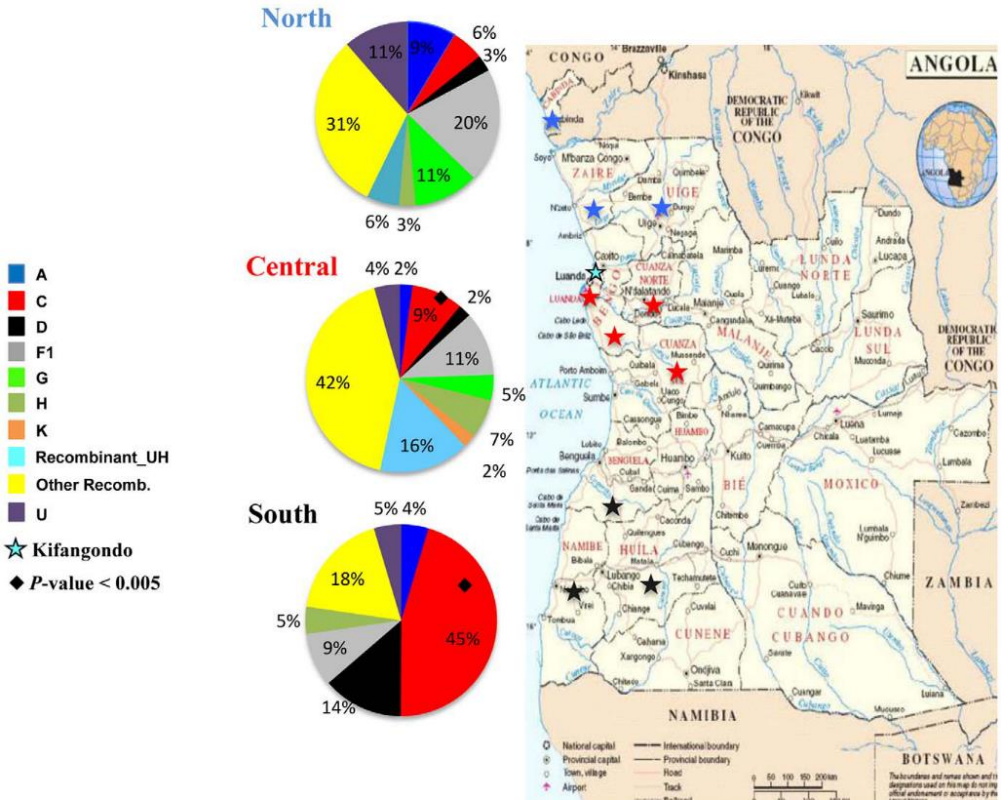


Figura 10 — Distribuição geográfica dos subtipos do VIH-1 em Angola. A distribuição dos subtipos do HIV-1 em Angola é muito diversificada com uma variação importante na prevalência de diferentes variantes genéticas entre as regiões norte, centro e sul de Angola. O subtipo F1 predomina a região norte com 20%. O subtipo C predomina a região sul com 45%. O recombinante UH predomina a região central com 16%. (Copiado da referência 110)

Algumas análises filogenéticas realizados com sequências de isolados do VIH-1 de diferentes regiões de Angola, mostraram que o subtipo F1 é oriundo da América do Sul e que provavelmente foi introduzido em Angola em 1959[114,115]. A circulação de todos os subtipos do VIH-1 em Angola (exceto o subtipo B), representa grandes implicações clínicas e para saúde pública, sobretudo para o diagnóstico molecular e serológico da infeção, o desenho de vacinas e a eficácia do TARV[116].

1.7.2 TARV e mutações de resistência em Angola

Angola é um dos países com acesso livre e universal dos ARVs que estão atualmente aprovados pela agência americana de regulamentação dos medicamentos, para o tratamento de pacientes infetados com o VIH/SIDA[28]. Em Angola, existem unidades de prevenção, tratamento, cuidados e apoio psicossocial integrados nas unidades sanitárias para atender os pacientes infetados com o VIH nas 18 províncias do país, garantindo o aconselhamento, tratamento com os ARVs e prevenção da transmissão vertical. As diretrizes para o tratamento nacional de pacientes adultos e adolescentes, recomenda combinação de dois ITRN (TDF+3TC, TDF+FTC, AZT+3TC, ABC+3TC ou ddI+3TC) e um ITRNN (EFV ou NVP) como parte dos esquemas de primeira linha, enquanto os IP têm sido usados preferencialmente como parte dos regimes de segunda linha ou se houver contraindicações e intolerância aos ITRNN (Tabela 2)[28]. Atualmente, mais de 14 000 pessoas estão em seguimento e tratamento com ARVs, dos quais grávidas e crianças expostas ao VIH[103].

Os estudos de epidemiologia molecular do VIH-1 indicam a circulação de inúmeras variantes resistentes aos principais ARVs usados em Angola[110–113]. As dificuldades socioeconómicas têm sido apontado como um dos fatores principais para a falta de adesão ao TARV e aumento da circulação de variantes com mutações de resistência[117]. As mutações M184V, G190A, K103N e Y181C são frequentemente identificados em pacientes angolanos expostos aos ARVs ou em pacientes sem histórico de exposição aos ARVs, o que preocupa a comunidade científica e clínica sobre a eficácia ao TARV nas diferentes regiões de Angola[110–113]. Os mesmos estudos indicam que existe a necessidade de reforçar a vigilância do VIH-1, para garantir o controlo da diversidade genética e garantir o controlo da circulação de vírus com mutações de resistência na população geral de infetados expostos ou não expostos aos ARVs em Angola[110].

CAPÍTULO 2: Objetivos

2.1 Objetivo geral

Estudar a variabilidade genética do VIH em mulheres grávidas de Luanda, com principal ênfase na epidemiologia molecular do VIH-1 e a testagem *in vitro* de novos compostos derivados do AZT quanto à sua capacidade para inibir a replicação do VIH-1.

2.2 Objetivos específicos

I. Determinar a prevalência do VIH, coinfecções e os determinantes sociodemográficos que contribuem para a disseminação de doenças infeciosas em mulheres grávidas de Luanda.

II. Investigar a diversidade genética do VIH-1 e mutações de resistência aos fármacos antirretrovirais em mulheres grávidas recém-diagnosticadas com VIH e *naïves* ao tratamento antirretroviral em Luanda.

III. Identificar os determinantes sociodemográficos que influenciam para a disseminação de variantes do VIH-1 com mutações de resistência aos fármacos antirretrovirais em mulheres grávidas de Luanda.

IV. Avaliar a eficácia de novos compostos sintetizados com modificação química no grupo 3'-Azido do AZT quanto a sua toxicidade e capacidade para inibir a replicação do VIH-1 *in vitro*.

CAPÍTULO 3: Publicações científicas

Manuscrito 1 (Publicado)

HIV, hepatitis B virus, hepatitis C virus, and syphilis among pregnant women attending antenatal care in Luanda, Angola: seroprevalence and risk factors

Received: 24 March 2020 | Accepted: 7 June 2020

DOI: 10.1002/jmv.26148

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

HIV, hepatitis B virus, hepatitis C virus, and syphilis among pregnant women attending antenatal care in Luanda, Angola: Seroprevalence and risk factors

Cruz S. Sebastião[1,2,3,4] | Zoraima Neto[1] | Domingos Jandondo[1] | Marinela Mirandela[1] | Joana Morais[1,2,5] | Miguel Brito[2,6]

[1]Molecular Biology Laboratory, Instituto Nacional de Investigação em Saúde, Luanda, Angola

[2]Department of Laboratory, Centro de Investigação em Saúde de Angola, Luanda, Angola

[3]Instituto Superior de Ciências da Saúde, Universidade Agostinho Neto, Luanda, Angola

[4]Laboratory of Immunobiology and Pathogenesis of CEDOC, NOVA Medical School, Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Lisboa, Portugal

[5]Faculdade de Medicina, Universidade Agostinho Neto, Luanda, Angola

[6]Health and Technology Research Center, Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Lisboa, Portugal

Correspondence
Miguel Brito, Health and Technology Research Center, Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Av. D. João II, Lote 4.69.01, 1990-096 Lisboa, Portugal.
Email: miguel.brito@estesl.ipl.pt

Funding information
Fundação para a Ciência e a Tecnologia, Grant/Award Number: SFRH/BD/135296/2017

Abstract

Infectious diseases during pregnancy remain a public health concern, especially in a resource-limited setting. The study aimed to determine the seroprevalence and determinants of HIV and co-infection with hepatitis B virus (HBV), hepatitis C virus (HCV), and syphilis among pregnant women attending antenatal care in Luanda, the capital city of Angola. A cross-sectional study was conducted with 1612 pregnant women screened for HIV during antenatal care. HIV-reactive were also screened for the HBV, HCV, and syphilis using immunoassay kits. A logistic regression model, adjusted odds ratios (AOR) and their 95% confidence interval (CI) were calculated with a level of significance set at 5%. The overall seroprevalence of HIV was 2.6%. About 13% of HIV-positive pregnant women were coinfected. From which, 7.5% were reactive to HBV and 5% to syphilis. There was no reactivity to HCV. Pregnant women younger aged than 25 years were significantly protected from HIV-infection (AOR, 0.43 [95% CI, 0.20-0.91], $P = .026$). The co-infection was 1.3 times (AOR, 0.04-41.0) in younger aged than 25 years, 7.0 times (AOR, 0.50-99.2) to residents in urbanized areas, and 1.4 times (AOR, 0.10-20.9) in pregnant women with a high educational level. In conclusion, infectious diseases are a public health burden among pregnant women in Luanda. However, include an integrated antenatal screening mainly in urbanized areas is crucial to reduce the spread of infectious diseases in different communities of Angola.

KEYWORDS

Angola, hepatitis B virus, hepatitis C virus, HIV infection, Luanda, pregnant women, syphilis

1 | INTRODUCTION

Infectious diseases during pregnancy remain a public health concern especially in low- and middle-income countries (LMIC).[1] Among the infections with importance in the morbidity and mortality rates, the human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and syphilis can be highlighted. Globally, an estimated 37.9 million people were living with HIV infection worldwide,[2] 257 million and 71 million people with HBV and HCV infection, respectively,[3] and 6 million new cases of syphilis are detected each year.[4] In a resource-limited setting, the screening of these infections during pregnancy may reduce the risk of vertical transmission to less than 5%, however, without intervention, the vertical transmission can be about 15% to 45%.[5] A previous study conducted in Luanda showed that the prevalence of HIV, HBV, and syphilis among pregnant women is 4.5%, 8.1%, and 5.4%, respectively.[6]

Infectious diseases during pregnancy play a significant role in maternal mortality rates and increase the risk of mortality among

neonates during the first weeks of life. However, understanding the factors related to infectious diseases would be beneficial for any program to prevent the spread of infections. Previous studies have reported that the lack of formal education, inadequate access to health care and poverty increase the risk of spread infectious diseases mainly in LMICs.[7] Limited information regarding determinants of the spread of infectious diseases is available among pregnant women from Luanda. Thus, the current study was conducted to estimate the seroprevalence and determinants related to infectious diseases in pregnant women attending antenatal care in Luanda, the capital city of Angola. This seroepidemiological study is critical in understanding and to strengthen the control of infectious diseases in the different communities of Angola.

2 | MATERIALS AND METHODS

2.1 | Study design and setting

The current study was part of a cross-sectional study carried out with 1612 pregnant women at all stages of pregnancy attending antenatal care at the Lucrecia Paim Maternity hospital during the months of April to June 2018, in Luanda, the capital city of Angola. This is a public hospital and reference center which provides health care for pregnant women and newborns from all provinces of Angola. The hospital team registered and codified of participant's identities and providing follow up care. The research team used a structured closed-ended questionnaire to obtain information regarding personal sociodemographic characteristics (age, local of residence, level of education, and occupation).

2.2 | Sample collection, molecular, and serological testing

The capillary puncture was performed and an estimated volume of 50 μL of the blood samples was collected with capillary tubes in each participant. An anti-HIV rapid test was performed at the health facility using the Determine HIV 1/2 Rapid Test (Alere, Japan). The rapid HIV test was considered positive when two lines of any intensity appeared in the control and patient areas, while tests with only one line in the control area and no lines in the patient area were considered negative. All tests that did not reveal any lines or did not reveal the line in the control area were considered invalid and the test was repeated with a new sample. The HIV-reactive pregnant women were retested with the rapid HIV test Unigold HIV (Trinity Biotech, Ireland), according to the national guidelines for HIV testing in Angola.[8] Pregnant women were considered HIV-positive when they presented independent positive results for both Determine and Unigold rapid HIV tests. There were no discordant results. A volume of 5 mL of intravenous whole blood was collected in a tube containing ethylenediaminetetraacetic acid (EDTA) in all HIV-positive pregnant women. The tubes containing the blood samples were centrifuged, the plasma was aliquoted and stored at −80°C until further analysis. Plasma samples were thawed and HIV ribonucleic acid (RNA) was manually extracted from 140 μL of plasma with the QIAamp Viral RNA kit (QIAGEN, Germany), according to the instructions provided by the manufacturer. The HIV-infection was further confirmed by nested polymerase chain reaction (PCR) using the protocol described previously.[9] Commercially available immunoassay rapid test kits were used to screen the presence of the HBV (Rapid Labs, UK), HCV (Rapid Labs), and Syphilis (Labmann). Briefly, a volume of 50 μL of plasma sample was used for each rapid test targeting the HBV, HCV, and Syphilis. Following the manufacturer's instructions, the rapid tests were considered positive when two lines appeared in the control and patient areas, while tests with only one line in the control area were considered negative. All tests that did not reveal any lines or did not reveal the line in the control area have been invalidated and the test repeated. No external controls (known as positive and negative) were included. The laboratory procedures, molecular and serological testing were performed at the molecular biology laboratory of the Instituto Nacional de Investigação em Saúde (INIS), in Luanda, Angola.

2.3 | Statistical analysis

The data were coded, entered, and analyzed in the Statistical Package for the Social Sciences (SPSS) version 25 (IBM SPSS Statistics). The frequencies and percentages were part of the descriptive analysis. Mean and the standard deviation (SD) were recorded to the data normally distributed. All variables were categorized and dichotomized to check potential interactions on the occurrence of infection. A univariate and multivariate logistic regression model was performed with all independent variables. The goodness of fit was based on the Hosmer-Lemeshow test. Odds ratio (OR) and their 95% confidence intervals (CIs) were calculated. The reported p-value are two-tailed and deemed statistically significant when $P < .05$.

2.4 | Ethical considerations

HIV test is offered in all pregnant women as part of the routine tests in antenatal screening public health programs. Ethical approval for this study was obtained from the Ethics Committee of Angola (reference number 13/2018) and the general directorate from Lucrecia Paim Maternity (reference number 083/GDG/MLP/2018). Moreover, verbal and written consent was secured from each pregnant woman, their parent or legal guardian after the objectives of the study were explained to them. The information from the participants and the clinical sample collected was used only for the stated objectives and kept confidential. Consent to participate was secured from all participants, their parent or legal guardian before being enrolled in the study, and agreed with the publication of the findings.

3 | RESULTS

3.1 | Sociodemographic characteristics

A total of 1612 pregnant women took part in this study. The general background of pregnant women is displayed in Table 1. Age ranged from 12 to 45 years. The mean age was 27 ± 7 years old. A greater number of the pregnant women (61.8%) were in the age group ≥25 years, living in a rural area (60.1%), with a high educational level (57.3%), and employed (54.5%). There were 102 (6.3%) pregnant women in their first trimester of gestational, 176 (10.9%) in their second trimester, and 1334 (82.8%) in their third trimester.

3.2 | Seroprevalence and determinants of HIV and co-infection

Forty-two pregnant women out of 1612 enrolled in this study were reactive against HIV antibodies, which represented an overall seroprevalence of 2.6%. Although no significant outcome was observed, multivariate analysis showed marginally increase in likelihood of contracting HIV infection in pregnant women residents in an urbanized area (AOR, 1.41 [95% CI, 0.76-2.60], $P = .276$), with a high educational level (AOR, 1.01 [95% CI, 0.53-1.95], $P = .969$), and employed (AOR, 1.03 [95% CI, 0.54-1.97], $P = .933$). On the other hand, both univariate and multivariate analyses showed a significant protective factor from HIV-infection in pregnant women younger aged than 25 years ($P < .05$) (Table 1).

The screened and respective determinants of co-infection were evaluated in 40/42 HIV-positive pregnant women. Due to the lack of screening kits, 2/42 HIV-positive pregnant women were not tested. Overall, 5/40 (12.5%) HIV-positive pregnant women were coinfected. From which, 3/40 (7.5%) were reactive to HBV and 2/40 (5%) were reactive to syphilis. None of these screened HIV-positive pregnant women were reactive to HCV and there was no triple infection. There was no significant outcome regarding sociodemographic characteristics and co-infection, but the likelihood to be coinfected in pregnant women younger aged than 25 years was 1.3 times (AOR, 0.04-41.0), residents in urbanized areas were 7.0 times (AOR, 0.50-99.2), and with a high educational level was 1.4 times (AOR, 0.10-20.9) (Table 2).

4 | DISCUSSION

HIV testing in pregnant women is a good indicator of the epidemic in the general population and has been used to predict the prevalence of young children.[10] Since the first case of HIV infection was reported in 1985 in Angola, the infection has spread rapidly to the general population from urbanized to rural areas.[11] Although there are studies with a comparatively high seroprevalence in pregnant women, as in Ethiopia (5.5%),[12] Tanzania (5.6%),[13] and Cameroon (6%),[14] the HIV prevalence in this study was slightly lower. In contrast, it is higher than reports in pregnant women from India (0.88%),[15] and Brazil (0.09%).[16] The variation in HIV prevalence across studies might be attributed to the socio-cultural differences, sexual behavior, and surveillance methodologies during antenatal care. Although our findings do not represent the general population from Luanda, it may partly reflect the effect of effective awareness national programs on the control of HIV infection in Luanda imposed in health services since 2004.[11]

Evidence supported that infectious diseases are associated with income since in LMICs there is less access to preventive information

TABLE 1 Seroprevalence and determinants of HIV infection among pregnant women attending antenatal care in Luanda, Angola, 2018

		HIV prevalence		Univariate analysis		Multivariate analysis[a]	
Characteristics	(%)	Negative (%)	Positive (%)	OR (95% CI)	*P*-value	AOR (95% CI)	*P*-value
Overall	1612 (100)	1570 (97.4)	42 (2.6)				
Age groups							
<25 y	615 (38.2)	606 (98.5)	9 (1.5)	0.43 (0.21-0.91)	.028*	0.43 (0.20-0.91)	.026*
≥25 y	997 (61.8)	964 (96.7)	33 (3.3)	1.00	…	1.00	…
Place of residence							
Rural	969 (60.1)	947 (97.7)	22 (2.3)	1.00	…	1.00	…
Urban	643 (39.9)	623 (96.9)	20 (3.1)	1.38 (0.75-2.55)	.302	1.41 (0.76 - 2.60)	.276
Education							
Low	688 (42.7)	670 (97.4)	18 (2.6)	1.00	…	1.00	…
High	924 (57.3)	900 (97.4)	24 (2.6)	0.99 (0.53-1.84)	.981	1.01 (0.53-1.95)	.969
Occupation							
Unemployed	733 (45.5)	714 (97.4)	19 (2.6)	1.00	…	1.00	…
Employed	879 (54.5)	856 (97.4)	23 (2.6)	1.01 (0.55-1.87)	.975	1.03 (0.54-1.97)	.933

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; OR, odds ratio.
[a]Adjusted for all the independent variables listed.
*Variables were significant ($P < .05$).

TABLE 2 Determinants of co-infection among HIV-infected pregnant women attending antenatal care in Luanda, Angola, 2018 (N = 40)

	HBV		Syphilis		Univariate analysis		Multivariate analysis[a]	
Characteristics	No (%)	Yes (%)	No (%)	Yes (%)	OR (95% CI)	*P*-value	AOR (95% CI)	*P*-value
Overall	37 (92.5)	3 (7.5)	38 (95.0)	2 (5.0)				
Age groups								
<25 y	7 (87.5)	1 (12.5)	8 (100)	0 (0.0)	1.00 (0.96-0.4)	1.000	1.33 (0.04-41.0)	.868
≥25 y	30 (93.8)	2 (6.3)	30 (93.8)	2 (6.3)	1.00	...	1.00	...
Place of residence								
Rural	19 (95.0)	1 (5.0)	20 (100)	0 (0.0)	1.00	...	1.00	...
Urban	18 (90.0)	2 (10.0)	18 (90.0)	2 (10.0)	4.75 (0.48-46.9)	.182	7.01 (0.50-99.2)	.150
Education								
Low	15 (88.2)	2 (11.8)	16 (94.1)	1 (5.9)	1.00	...	1.00	...
High	22 (95.7)	1 (4.3)	22 (95.7)	1 (4.3)	0.44 (0.07-3.01)	.406	1.44 (0.10-20.9)	.790
Occupation								
Unemployed	15 (83.3)	3 (16.7)	16 (88.9)	2 (11.1)	1.00	...	1.00	...
Employed	22 (100)	0 (0.0)	22 (100)	0 (0.0)	0.0 (0.0-0.0)	.998	0.0 (0.0-0.0)	.998

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; HBV, hepatitis B virus; OR, odds ratio.
[a]Adjusted for all the independent variables listed.

and healthcare.[1] Moreover, the inequality of resource distribution in the population unemployed and with low educational levels lead to risky sexual behavior and an increase in infectious diseases. In our multivariate analysis, there is evidence that the overall HIV prevalence in pregnant women from Luanda is related to advanced aged, local of residence, educational levels, and occupation. Moreover, favorable results to HIV infection were observed in pregnant women younger aged than 25 years (AOR, 0.43, $P = .026$) (Table 1). In contrast, pregnant women older-aged than 24 years from Cameroon,[17] South Africa,[18] and Rwanda,[19] were more likely to be infected with HIV. Increased HIV prevalence in adult women may have been exacerbated by low education, low access to health care, and unemployment. We found that pregnant women resident in urbanized areas, with a low educational level, and unemployed are less likely to visit an antenatal hospital, making these a vulnerable group for infectious diseases (Table 1). Consistent with our results, high HIV prevalence was reported in pregnant women from urbanized areas in the Democratic Republic of the Congo,[20] and Tanzania,[13] but different results were reported in Rwanda,[19] Cameroon,[17] and Ethiopia.[21] In contrast with our results, low HIV prevalence was observed in pregnant women with a high educational level in the USA,[22] and China.[23]

The high prevalence of HIV/HBV co-infection observed is not surprising since the HIV/HBV co-infection in sub-Saharan Africa is estimated at 6% to 25%.[24] However, a high HIV/HBV co-infection in pregnant women suggests a potential source for the spread of viral infections in Luanda. Our findings are consistent with those reported in HIV-positive pregnant women from Cameroon (7.7%),[25] higher than to reported in Nigeria (0.5%),[26] Botswana (3.1%),[27] Rwanda (4.1%),[19] and Sudan (5.6%),[28] but is lower than that reported in Ethiopia (12.1%),[12] and Ghana (14.9%).[29] The proportion of HIV/HCV co-infection observed in our cohort was compared to reported in southern (3.3%) and north (42.3%) of sub-Saharan Africa,[30] and European countries (12.3%).[31] Most of the time, high HIV/HBV co-infection is attributed to the fact they share mutual routes of transmission. The low prevalence of HCV in our study may be explained by the fact these infections were evaluated exclusively in HIV-infected pregnant women or by the effectiveness of serological rapid diagnostic tests. On the other hand, the prevalence of syphilis was high to that reported in HIV-positive pregnant women from Uganda (0.52%)[32] and Tanzania (0.9%).[33]

Although there was no evidence to suggest a significant association, it is worth noting that most cases of co-infections were observed in pregnant women older-aged than 24 years, from urbanized areas, with low educational and unemployed (Table 2). The high concentration of key populations such as sex workers, injecting drug users and men who have sex with men in urbanized settings may explain the high risk of the spread of infectious diseases in urbanized areas compared to the rural areas. Our findings suggest that efforts to prevent the spread of infectious diseases should begin in urbanized areas and expand into rural areas. Additionally, HBV vaccination strategies and screen of syphilis should be widely applied regardless of HIV prevention strategies.

This study has some potential limitations. First, the study was cross-sectional which limited the ability to examine causal relationships and not entirely represent the whole population in Luanda or pregnant women in other regions from Angola. Besides that, out of forty-two HIV-positive pregnant women, two were not screened for co-infection due to the lack of serologic tests at the time of the research, and the lower number of coinfected pregnant women limit a robust analysis. Second, HBV, HCV, and syphilis infection have been evaluated only in HIV-positive pregnant women. Third, a possible underestimation of the prevalence of the

evaluated markers should be considered since rapid diagnostic tests less sensitive than enzyme-linked immunosorbent assay (ELISA) or PCR were used to screen HBV, HCV, and syphilis. Fourth, HIV-RNA viral load and detection of other important markers of HBV, HCV, and syphilis were not determined because of the lack of laboratory setup. Despite these shortcomings, our findings provide important data on the epidemiology of infectious diseases in pregnant women from Luanda. Thus, further studies using ELISA or PCR tests for screening infectious diseases should be conducted in the larger population of different groups and communities of Angola. Besides, further studies on demographic and behavioral risk factors (eg, clinical/medical related factors) that influence the emergence and spread of infectious diseases in pregnant women should be carried out in Angola.

In summary, this study showed that HIV, HBV, and syphilis infections are important public health issues in pregnant women from Luanda that need to be addressed, while HCV infection does not seem to be the public health burden particularly among women living with HIV infection. Older-aged, urbanized areas, low educational levels, and unemployed were vulnerable groups. Our findings suggest that is crucial an integrated screened during antenatal care to avoid maternal transmission and the eventual adverse effects on neonates in Luanda. Moreover, health education about the routes of transmission and prevention should be given to reduce the spread of infectious diseases, particularly in pregnant women from urbanized settings.

ACKNOWLEDGMENTS

We would like to express our gratitude to the pregnant women for their participation, as well as collaborating with staff of INIS, INLS, ISCISA/Agostinho Neto University, and Lucrecia Paim Maternity. CSS was awarded a PhD scholarship (grant number SFRH/BD/135296/2017) from the PGCD and the FCT. Moreover, the contributions of Dr. Castigo Levita, Moisés Dembo, Pedro Castelo, Maria Afonso, Idalina Luamba, Ludovina Bartolomeu, Ricardo Mucusa, and Valter Nuaila are duly acknowledged. This study has been supported by a PhD grant from the Postgraduate Science for Development Program (PGCD) and the Foundation for Science and Technology (FCT), awarded to CSS (grant number SFRH/BD/135296/2017). The supporting institutions had no role in the design of the study and data collection, analysis, interpretation of data, preparation of the manuscript, or decision to publish.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

CSS, JM, and MB had full access to all of the data in the study. CSS, JM, and MB take responsibility for the study concept, design, integrity of the data, and the accuracy of the data analysis. CSS performed serological and molecular screening. CSS, DJ, and MM contributed to mobilization, data collection, and technical support. JM, ZN, and MB contributed to administrative support, supervised data collection, and fieldwork. CSS drafted the original draft. CSS, MB, and JM were responsible for statistical analysis and interpretation. CSS, MB, JM, and ZN conducted a critical revision of the manuscript for intellectual content. All authors read and approved the final manuscript for publication.

ORCID

Miguel Brito http://orcid.org/0000-0001-6394-658X

REFERENCES

1. Hogben M, Leichliter JS. Social determinants and sexually transmitted disease disparities. *Sex Transm Dis*. 2008;35(suppl):S13-S18. https://doi.org/10.1097/olq.0b013e31818d3cad
2. Unaids. Unaids Data 2019. Unaids. 2019.
3. WHO. Executive summary - Global hepatitis report, 2017. World Heal Organ. 2017.
4. Kojima N, Klausner JD. An update on the Global Epidemiology of Syphilis. *Curr Epidemiol Rep*. 2018;5:24-38. https://doi.org/10.1007/s40471-018-0138-z
5. WHO. Mothe-To-Child Transmission Of HIV, 1-2. 2017.
6. World Health Organization. Surveillance carried out in six provinces shows that hiv spread in Angola can be controlled. 2003:1-2.
7. Bärnighausen T, Hosegood V, Timaeus IM, Newell M. The socio-economic determinants of HIV incidence: evidence from a longitudinal, population-based study in rural South Africa. *Victoria*. 2010;21(suppl 7):1-17. https://doi.org/10.1097/01.aids.0000300533.59483.95
8. National Institute of Fighting against AIDS. Normas De Tratamento Antirretroviral. 2015:159.
9. Sebastião CS, Neto Z, de Jesus CS, et al. Genetic diversity and drug resistance of HIV-1 among infected pregnant women newly diagnosed in Luanda, Angola. *PLoS One*. 2019;14:1-10. https://doi.org/10.1371/journal.pone.0225251
10. Zaba B, Boerma T, White R. Monitoring the AIDS epidemic using HIV prevalence data among young women attending antenatal clinics: prospects and problems. *AIDS*. 2000;14(11):1633-1645. https://doi.org/10.1097/00002030-200007280-00020
11. National Institute of Fighting against AIDS. Plano Estratégico Nacional Para o Controlo das Infecções de Transmissão Sexual, VIH e SIDA Instituto Nacional de Luta Contra a Sida. 2006.
12. Kassa D, Gebremichael G, Tilahun T, et al. Prevalence of sexually transmitted infections (HIV, hepatitis B virus, herpes simplex virus type 2, and syphilis) in pregnant women in Ethiopia: trends over 10 years (2005-2014). *Int J Infect Dis*. 2019;79:50-57. https://doi.org/10.1016/j.ijid.2018.11.009
13. Manyahi J, Jullu BS, Abuya MI, et al. Prevalence of HIV and syphilis infections among pregnant women attending antenatal clinics in Tanzania, 2011 disease epidemiology-infectious. *BMC Public Health*. 2015;15(1):1-9. https://doi.org/10.1186/s12889-015-1848-5
14. Dionne-Odom J, Mbah R, Rembert NJ, et al. Hepatitis B, HIV, and syphilis seroprevalence in pregnant women and blood donors in Cameroon. *Infect Dis Obstet Gynecol*. 2016;2016:1-8. https://doi.org/10.1155/2016/4359401
15. Gupta S, Gupta R, Singh S. Seroprevalence of HIV in pregnant women in North India: a tertiary care hospital based study. *BMC Infect Dis*. 2007;7:4-8. https://doi.org/10.1186/1471-2334-7-133
16. Costa ZB, Machado GC, Avelino MM, et al. Prevalence and risk factors for Hepatitis C and HIV-1 infections among pregnant women in Central Brazil. *BMC Infect Dis*. 2009;9:1-9. https://doi.org/10.1186/1471-2334-9-116
17. Anoubissi JdeD, Gabriel EL, Nde CK, et al. Factors associated with risk of HIV-infection among pregnant women in Cameroon: evidence from the 2016 National Sentinel Surveillance Survey of HIV and syphilis. *PLoS One*. 2019;14(4):1-10. https://doi.org/10.1371/journal.pone.0208963

18. Chetty T, Vandormael A, Thorne C, Coutsoudis A. Incident HIV during pregnancy and early postpartum period: a population-based cohort study in a rural area in KwaZulu-Natal, South Africa. *BMC Pregnancy Childbirth*. 2017;17(1):1-10. https://doi.org/10.1186/s12884-017-1421-6
19. Mutagoma M, Balisanga H, Malamba SS, et al. Hepatitis B virus and HIV co-infection among pregnant women in Rwanda. *BMC Infect Dis*. 2017;17(1):1-7. https://doi.org/10.1186/s12879-017-2714-0
20. Mpody C, Thompson P, Tabala M, et al. Hepatitis B infection among pregnant and post-partum women living with HIV and on antiretroviral therapy in Kinshasa, DR Congo: a cross-sectional study. *PLoS One*. 2019;14(5):e0216293. https://doi.org/10.1371/journal.pone.0216293
21. Endris M, Deressa T, Belyhun Y, Moges F. Seroprevalence of syphilis and human immunodeficiency virus infections among pregnant women who attend the University of Gondar Teaching Hospital, Northwest Ethiopia: a cross sectional study. *BMC Infect Dis*. 2015;15(1):1-7. https://doi.org/10.1186/s12879-015-0848-5
22. Zahedi-Spung L, Young M, Haddad LB, Badell ML. Perceived barriers to antepartum HIV medication adherence in HIV infected pregnant women. *Infect Dis Obstet Gynecol*. 2018;2018:1-5. https://doi.org/10.1155/2018/4049212
23. Yang S, Yang C, Liao Q, et al. Analysis of HIV prevalence among pregnant women in Liangshan Prefecture, China, from 2009 to 2015. *PLoS One*. 2017;12(9):1-11. https://doi.org/10.1371/journal.pone.0183418
24. Stockdale AJ, Geretti AM. Chronic hepatitis B infection in sub-Saharan Africa: a grave challenge and a great hope. *Trans R Soc Trop Med Hyg*. 2015;109(7):421-422. https://doi.org/10.1093/trstmh/trv044
25. Sone LHE, Voufo RA, Dimodi HT, et al. Prevalence and identification of serum markers associated with vertical transmission of Hepatitis B in pregnant women in Yaounde, Cameroon. *Int J MCH AIDS*. 2017;6(1):69-74. https://doi.org/10.21106/IJMA.174
26. Opaleye OO, Igboama MC, Ojo JA, Odewale G. Seroprevalence of HIV, HBV, HCV, and HTLV among pregnant women in Southwestern Nigeria. *J Immunoass Immunochem*. 2016;37(1):29-42. https://doi.org/10.1080/15321819.2015.1040160
27. Mbangiwa T, Kasvosve I, Anderson M, et al. Chronic and occult hepatitis B virus infection in pregnant women in Botswana. *Genes*. 2018;9(5):1-13. https://doi.org/10.3390/genes9050259
28. Elsheikh RM, Daak AA, Elsheikh MA, Karsany MS, Adam I. Hepatitis B virus and hepatitis C virus in pregnant Sudanese women. *Virol J*. 2007;4:8-10. https://doi.org/10.1186/1743-422X-4-104
29. Frempong MT, Ntiamoah P, Annani-Akollor ME, et al. Hepatitis B and C infections in HIV-1 and non-HIV infected pregnant women in the Brong-Ahafo Region, Ghana. *PLoS One*. 2019;14(7):1-13. https://doi.org/10.1371/journal.pone.0219922
30. Loarec A, Carnimeo V, Molfino L, et al. Extremely low hepatitis C prevalence among HIV co-infected individuals in four countries in sub-Saharan Africa. *AIDS*. 2019;33(2):353-355. https://doi.org/10.1097/QAD.0000000000002070
31. Landes M, Newell ML, Barlow P, et al. Hepatitis B or hepatitis C coinfection in HIV-infected pregnant women in Europe. *HIV Med*. 2008;9(7):526-534. https://doi.org/10.1111/j.1468-1293.2008.00599.x
32. Taremwa IM, Twelwanike A, Mwambi B, Atuhairwe C. Laboratory assessment of SD Bioline HIV/Syphilis Duo Kit among pregnant women attending antenatal clinic Mayuge Health Center III, East central Uganda. *BMC Res Notes*. 2019;12(1):238. https://doi.org/10.1186/s13104-019-4272-6
33. Lawi JD, Mirambo MM, Magoma M, et al. Sero-conversion rate of syphilis and HIV among pregnant women attending antenatal clinic in Tanzania: a need for re-screening at delivery. *BMC Pregnancy Childbirth*. 2015;15(1):1-7. https://doi.org/10.1186/s12884-015-0434-2

How to cite this article: Sebastião CS, Neto Z, Jandondo D, Mirandela M, Morais J, Brito M. HIV, hepatitis B virus, hepatitis C virus, and syphilis among pregnant women attending antenatal care in Luanda, Angola: Seroprevalence and risk factors. *J Med Virol*. 2020;1–6. https://doi.org/10.1002/jmv.26148

Manuscrito 2 (Submetido para publicação)

Dengue virus among HIV-infected pregnant women attending antenatal care in Luanda, Angola: An emerging public health concern

Dengue virus among HIV-infected pregnant women attending antenatal care in Luanda, Angola: an emerging public health concern

Running title: **Dengue among HIV-infected pregnant women in Angola**

Cruz S. Sebastião[1,2,3,4,5], Zoraima Neto[2], Domingos Jandondo[2], Marinela Mirandela[2], Joana Morais[1,2,6] & Miguel Brito[1,7,*]

Author affiliations:

[1] *Centro de Investigação em Saúde de Angola, Luanda, Angola*

[2]*Instituto Nacional de Investigação em Saúde, Luanda, Angola*

[3]*Instituto Superior de Ciências da Saúde, Universidade Agostinho Neto, Luanda, Angola*

[4] *CEDOC, NOVA Medical School, Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Lisboa, Portugal*

[5]*Programa de Pós-Graduação Ciência para o Desenvolvimento, IGC, Lisboa, Portugal*

[6]*Faculdade de Medicina, Universidade Agostinho Neto, Luanda, Angola*

[7]*Health and Technology Research Center, Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Lisboa, Portugal*

Correspondence Author: Miguel Brito, Health and Technology Research Center, Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Lisboa, Portugal. E-mail: miguel.brito@estesl.ipl.pt

Cruz S. Sebastião ORCID – 0000 – 0003 – 1232 – 0119

Zoraima Neto ORCID – 0000 – 0003 – 1606 – 9996

Miguel Brito ORCID – 0000 – 0001 – 6394 – 658X

Abstract

OBJECTIVE: The spread of dengue virus (DENV) in endemic regions with HIV is a public health concern with greater importance when there is evidence of vertical transmission of DENV during pregnancy. Herein, we investigated DENV among HIV-infected pregnant women in Luanda, Angola.

METHODS: A total of 42 plasma samples from HIV-positive pregnant women were screened for DENV with RT-PCR and ELISA. Chi-square and logistic regression analysis were used to check interactions between DENV infection and patient demographics.

RESULTS: None of the specimens tested positive for DENV by RT-PCR. In what concerns seroprevalence, 94.4% of the samples were positive for IgG and 11.1% for IgM. Recent infection (IgG-/IgM+ or IgG+/IgM+) was detected in 11.1% of the samples, non-infection (IgG-/IgM-) in 5.6% and past infection (IgG+/IgM-) in 83.3%. Recent infection was high in pregnant women over 25 years old [OR: 13.0 (95% CI: 1.14-148), p=0.039].

CONCLUSIONS: The present study showed a high seroprevalence of DENV infection with laboratory evidence of a recent DENV infection among HIV-infected pregnant women attending antenatal care in Luanda province. This study provides critical data regarding the seroprevalence of DENV infection among HIV-infected pregnant women in Luanda. Future studies involving a larger sample size of HIV-infected pregnant women are necessary to support ongoing public health programs to combat arboviruses in endemic areas of Angola.

Keywords: dengue virus; HIV; seroprevalence; pregnant women; Angola

Introduction

Dengue is a mosquito-borne viral infectious disease caused by the dengue virus (DENV) transmitted to humans through the *Aedes* mosquitoes. It is endemic in more than 100 tropical and subtropical countries in Southeast Asia, the Americas, the western Pacific, Africa, and the eastern Mediterranean regions (1). It is estimated 10 000 deaths and 390 million DENV infections occur per year, of which 96 million infected people remain asymptomatic or subclinical (2,3). Phylogenetic analysis indicated that the DENV lineage circulating in Angola, since at least 1968, belongs to the American-African one, and strongly suggesting that DENV is endemic in Angola (4). During the years 2013 and 2018, Angola experienced a large DENV outbreak that was concentrated in Luanda province, the capital city of Angola (4,5). A large proportion of the population from Luanda live in slums and tenements housings with limited access to health care and poor basic sanitation. In addition, the Luanda province is intensely visited by international business travelers mostly because of the oil trade.

Many viral infectious diseases coexist in tropical and subtropical regions which have contributed to the high rates of morbidity and mortality in high-risk populations such as pregnant women. The determinants of the pathogenesis of DENV infection among HIV-infected patients are not fully understood (6–9). Previous studies showed that DENV infection during pregnancy is related to vertical transmission, miscarriage, and mother mortality (9–13).

Limited published studies assessed DENV infection among HIV-infected pregnant women, as well as the consequences for their infants in Angola. In the present study, we aimed to investigate the seroprevalence of DENV infection among HIV-infected pregnant women attending antenatal care in Luanda province. It is our purpose to support public health programs on prevention and elimination of the risk factors of adverse pregnancy outcomes in HIV-infected pregnant women from endemic areas with DENV in Angola.

Methods

Study design and setting

The current study was part of a cross-sectional study carried out with 42 pregnant women newly diagnosed with HIV regardless of the gestational period. All the subjects were attending antenatal care at the Lucrecia Paim Maternity hospital from April to June 2018, in Luanda province, the capital city of Angola. The Lucrecia Paim Maternity is a tertiary health unit and a reference in research, training, and antenatal care for pregnant women from all provinces of Angola. The HIV-infected pregnant women were interviewed, after signed informed consent, and a structured questionnaire was used to obtain personal demographic characteristics as age, local residence, level of education, occupation, and gestational period.

Sample collection and molecular testing

Plasma samples separated from a 5mL intravenous blood sample obtained from each participant were stored at -80°C until further analysis. The total viral ribonucleic acid (RNA) was manually extracted from 140µL of plasma samples using the QIAamp Viral RNA kit (QIAGEN, Germany) following the manufacturer's instructions. The HIV infection was confirmed by nested polymerase chain reaction (PCR) using the protocol described previously (14). The presence of the DENV RNA was screened using real-time reverse-transcription polymerase chain reaction (RT-PCR) with the Applied Biosystems 7500 Fast RT-PCR System (Thermo Fisher Scientific). The Centers for Disease Control and Prevention (CDC) Trioplex real-time RT-PCR assay was used (15,16). Briefly, the RT-PCR was carried out using 10µL of the RNA in a final reaction volume of 25µL containing primers and dual-labelled hydrolysis (TaqMan®) probes targeting *in vitro* qualitative detection of the DENV, Zika virus (ZIKV), and Chikungunya virus (CHIKV). Cycling conditions for the RT-PCR consisted of 30 minutes at 50°C followed by 45 cycles of 2 minutes at 95°C, 15 seconds at 95°C and 1 minute at 60°C. The fluorescence capture was set to detect light emissions by the DENV, ZIKV, and CHIKV

through the fluorescent dye FAM, Texas Red, and VIC, respectively. The amplification curves were evaluated individually for each target virus and the threshold line was placed above the beginning of the exponential phase of the curve. Positive and negative control samples were included. The results of RT-PCR were considered valid when positive control samples showed a cycle threshold (CT) value below 31. Specimens with CT values below 31 were considered positive while specimens with CT values equal or above 31 were considered negative.

Serological testing

Commercially available indirect enzyme-linked immunosorbent (ELISA) assay was used to screen the presence of immunoglobulin M (IgM) (EUROIMMUN, Germany) and immunoglobulin G (IgG) (EUROIMMUN, Germany) antibodies against DENV infection following the manufacturer's instructions. Briefly, 100µL of 1:100 diluted plasma samples were added to wells coated with anti-human IgM or IgG and incubated at 37°C. After that, the wells were washed three times and incubated at room temperature (RT) with 100µL of the conjugate. Then, the wells were again washed three times and incubated at RT in the dark with 100µL of the substrate. Finally, 100µL of the stop solution was added and the plaque was read ten minutes later. The absorbance value was measured at 450 and 620 nm. Positive and negative control were included in each ELISA assay. A positive ELISA result was defined as an absorbance twice that of the negative control according to the manufacturer's instructions. Besides, the serological results of DENV infection were grouped as follows: non-infection (IgG-/IgM-), past infection (IgG+/IgM-), and recent infection (IgG-/IgM+ or IgG+/IgM+). The molecular and serological testing was performed at the molecular biology laboratory of the Instituto Nacional de Investigação em Saúde (INIS), in Luanda, Angola.

Statistical analysis

The data were coded and analyzed using the Statistical Package for the Social Sciences (SPSS) version 25 for windows (IBM SPSS Statistics, USA). The descriptive analysis was presented as

frequencies and percentages. The data normally distributed were presented as mean and the standard deviation. The patient demographics were categorized and dichotomized as follows: age (<25 years old vs. ≥25 years old), local residence (urbanized area *vs.* rural area), level of education (pregnant women illiterate and with primary education were categorized as a low educational level while pregnant women with secondary or tertiary levels were categorized as high educational level), occupation (unemployed *vs.* employed), and gestational period (first trimester *vs.* second or third trimester). A Chi-square test and logistic regression analysis were carried out with all explanatory variables to check the potential interactions between patient demographics and DENV infection in HIV-infected pregnant women. Odds ratio (OR) and their 95% confidence intervals (CIs) were calculated to determine the strength and direction of the interaction between variables. The reported p-value are two-tailed and deemed statistically significant when $p<0.05$.

Ethics approval

The study protocol was reviewed and ethical approval was obtained from the Ethics Committee of Angola (nr.13/2018) and the general directorate from Lucrecia Paim Maternity hospital (nr.083/GDG/MLP/2018). All HIV-infected pregnant women undergoing an antenatal care examination were invited and consent to participate was secured from each participant or legal guardians.

Results

Between April to June 2018, a period of a DENV outbreak in Luanda province, 42 HIV-infected pregnant women were enrolled in our study. The ages of HIV-infected pregnant women ranged from 14 to 42 years old. The mean age was 28±6 years old. The majority of pregnant women were in the age group ≥25 years (78.6%, 33/42), living in a rural area (52.4%, 22/42), with a low educational level (85.7%, 36/42), and unemployed (64.3%, 27/42). When the pregnant women

were invited and their blood samples were drawn, there were 9 (21.4%) in their first trimester of gestation, 11 (26.2%) in their second trimester of gestation, and 22 (52.4%) in their third trimester of gestation.

DENV infection among HIV-infected pregnant women

The results of nested-PCR confirmed HIV infection in all specimens collected. A CT value of 30.2, 24.0, and 26.6 was obtained from RT-PCR in the positive control samples used for target DENV, ZIKV, and CHIKV, respectively. None of the 42 samples subjected to RT-PCR tested positive for DENV, ZIKV, or CHIKV. The presence of IgG and IgM antibodies against DENV infection could only be screened in 36 plasma samples out of 42 specimens collected due to a lack of plasma samples. The demographic characterization and seroprevalence of DENV infection among HIV-infected pregnant women enrolled in this study are shown in Table 1. The overall seroprevalence of DENV infection detected by ELISA among HIV-infected pregnant women was 94.4%. A total of 94.4% (34/36) and 11.1% (4/36) of plasma samples were IgG and IgM positive, respectively. Recent infection (IgG-/IgM+ or IgG+/IgM+) was detected in 11.1% (4/36) of the plasma samples whereas non-infection (IgG-/IgM-) and past infection (IgG+/IgM-) were detected in 5.6% (2/36) and 83.3% (30/36), respectively (Table 1).

Determinants of DENV infection among HIV-infected pregnant women

Significant differences were observed between recent infection and age groups, being more frequent in under 25 years old age class (Table 1). Non-infection and past infection were significantly associated with the gestational period ($p<0.05$). On the other hand, there were no significant differences when compared IgG or IgM seropositive between the place of residence, educational level, and occupation (Table 1). The chances of recent infection were higher in HIV-infected pregnant women younger than 25 years old [OR: 13.0 (95% CI: 1.14-148), p=0.039] and in the first trimester of gestation [OR: 4.3 (95% CI: 0.50-37.3), p=0.182] (Table 2). On the other hand,

168 despite no statistical significance observed, all recent DENV infection was observed in pregnant
169 women with low educational level and unemployed (Table 1). Moreover, a putative chance related
170 to DENV infection has been observed in low educational level, unemployed pregnant women, and
171 pregnant women living in a rural area (Table 2).
172

173 ## Discussion

174 Although DENV and HIV infections are endemic and major public health problem in Angola, there is
175 a lack of published studies assessing the seroprevalence of DENV fever in HIV-infected patients in
176 Angola. To the best of our knowledge, this seroprevalence study is the first description of DENV
177 infection among HIV-infected pregnant women in Luanda province. In this analysis of DENV
178 infection in HIV-infected pregnant women during the recent DENV outbreak in the year 2018, the
179 overall seropositivity was 94.4%. Almost all HIV-infected pregnant women (83.3%, 30/36) had
180 laboratory evidence of past DENV infection whereas 11.1% (4/36) had recent DENV infection (Table
181 1). The recent DENV outbreak in Luanda could have contributed to the high seropositivity found in
182 this studied population (5).
183 The negative results observed in IgM positive samples (4/36) on the detection of viral DENV RNA by
184 RT-PCR in our study, could be ascribed to the fact that IgM antibodies could be detected after the 7
185 days of onset of symptoms as well as two to three months after viral exposure whereas the DENV
186 viremia period is short (about five days) indicating that the investigation of recent DENV infection
187 through serological markers (IgM/IgG) may not be feasible in endemic regions with a large number
188 of asymptomatic patients (17).
189 The high level of DENV IgG positive (94.4%, 34/36) observed in our study, could suggest the
190 previous infection by DENV in almost all HIV-infected pregnant women. Moreover, our results also
191 indicated that Luanda province is an endemic region with DENV in Angola with a high risk of the

192 spread of infectious diseases transmitted by an arthropod. Similar results of a high level of DENV
193 IgG positive were also observed in 92% of the HIV-infected pregnant women screened for DENV
194 infection in Brazil (18), whereas different results were found in pregnant women from the
195 Democratic Republic of Sao Tome and Principe (19) and China (20). The unprecedented population
196 growth, uncontrolled urbanization, house-to-house movements of peoples living in precarious
197 conditions of basic sanitation, international trade, travel of people for countries with active DENV
198 transmission, climate change, breach of public health infrastructure, and breach of vector control
199 programs, contribute disproportionately to spread of local and imported cases of DENV in Angola
200 (1,21).
201 The level of recent DENV infection (IgM positive - 11.1%) identified in our study was high compared
202 to the previous report in pregnant women (2.8 - 10.6%) from Brazil (17,22). Although adult
203 pregnant women are the population most affected by HIV infection (75.0%, 27/36) (Table 1), a
204 statistically significant risk factor for DENV infection was observed in younger HIV-infected pregnant
205 women [OR: 13.0 (95% CI: 1.14-148), p=0.039] (Table 2). One of the possible explanations for the
206 high likelihood of recent DENV infection among younger pregnant women in Luanda could be
207 attributed to the fact that these young women work or have more outdoor activities, and therefore
208 increasing exposure to the *Aedes* mosquitoes that causes DENV infection. These results are not
209 surprising since 91.7% and 69.4% of the screened HIV-infected pregnant women had a low
210 academic level and were unemployed, respectively (Table 1).
211 Previous studies have shown an increase in incidence, hospitalization, and severity of DENV
212 infection among younger patients over the past ten years indicating a possible change in the
213 population at risk of infection, from adults to younger people (23). This epidemiological change and
214 the high risk of recent DENV infection in younger patients observed in our study could be explained
215 by the fact that adults develop immune responses to all dengue serotypes over time making

younger people a group susceptible to DENV infection, however, these changes should serve as a warning to Angolan health authorities to provide timely health care for younger patients. We also cannot exclude the possibility that DENV infection leads to an increase in miscarriage risk or even mother mortality, and in older age classes these events could be more frequent, leading to a reduction in successful pregnancies. Indeed, other studies have shown a higher risk of miscarriage (12) or higher mortality (13) with DENV infection during pregnancy.

Although no statistical significance was observed, the majority of recent DENV infections (75.0%, 3/4), as well as the chances of recent infection, were identified among HIV-infected pregnant women living in rural areas from Luanda. On the other hand, the multivariate analysis showed that the recent DENV infection is 7.38 times (95% CI: 0.43 - 126) higher among HIV-infected pregnant women in their first trimester of gestation compared to pregnant women in their second or third trimester of gestation (Table 2). Once again, miscarriage or mother mortality can be a possible explanation for these results. Our findings and the recent evidence of vertical transmission, miscarriage, and mother mortality of DENV infection during pregnancy (10–13), indicate that continuous surveillance which includes differential screening for the acute febrile syndrome among pregnant women to prevent adverse effects and vertical transmission of DENV infection in the non-urbanized regions should be considered mainly during periods of increased viral circulation in endemic areas from Angola.

Our study has some potential limitations. The limited representativeness of the studied population diminishes the strength of our results and might not be sufficient to support public health programs on the risk factors of vertical transmission and adverse pregnancy outcomes of DENV infection among HIV-infected pregnant women in Luanda. Besides, were not evaluated specific non-structural proteins (e.g., NS1) to indicate recent DENV infection, as well as the possibility of cross-reactivity between assays based on the detection of antibodies against different flaviviruses such as

ZIKV, a flavivirus closely related to DENV (24). Despite these limitations, our results highlight the concern about the risk of asymptomatic DENV infection during pregnancy suggesting that the Angolan Ministry of Health should consider the possibility of implementing screening and monitoring programs for DENV infections in HIV-infected pregnant women in the future, especially in endemic areas for DENV in Angola (e.g., provinces of Luanda and Cuanza Norte). Future large-scale prospective studies with extensive laboratory testing of DENV infection in HIV-infected pregnant women are necessary, to obtain epidemiological and clinical data that could elucidate the effect of asymptomatic or severe DENV infection on the population of HIV-infected pregnant women in endemic areas of Angola.

Conclusion

The present study showed a high seroprevalence of DENV infection with laboratory evidence of a recent DENV infection among HIV-infected pregnant women attending antenatal care in Luanda province. This study provides critical data regarding the seroprevalence of DENV infection among HIV-infected pregnant women in Luanda. Future studies involving a larger sample size of HIV-infected pregnant women are necessary to support ongoing public health programs to combat arboviruses in endemic areas of Angola.

Acknowledgments

We are grateful to the HIV-infected pregnant women for their participation, as well as the institutional support of INIS, ISCISA/Agostinho Neto University, and Lucrecia Paim Maternity. A Ph.D. scholarship from the Programa de Pós-Graduação Ciência para o Desenvolvimento (PGCD)

and the Fundação para a Ciência e a Tecnologia (FCT), Portugal, was awarded to CSS (SFRH/BD/135296/2017).

Conflict of interest

The authors declare no conflict of interest.

References

1. Guzman MG, Harris E. Dengue. Lancet. 2015;385(9966):453–65.
2. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013;496(7446):504–7.
3. Messina JP, Brady OJ, Golding N, Kraemer MUG, Wint GRW, Ray SE, et al. The current and future global distribution and population at risk of dengue. Nat Microbiol. 2019;4(9):1508–15.
4. Ministry of Health of Angola. Ongoing Dengue Epidemic — Angola, June 2013. MMWR. 2013;62(24):504–7.
5. Hill SC, Vasconcelos JN de, Granja BG, Thézé J, Jandondo D, Neto Z, et al. Early Genomic Detection of Cosmopolitan Genotype of Dengue Virus Serotype 2, Angola, 2018. Emerg Infect Dis. 2019;25(4):2017–20.
6. Watt G, Kantipong P, Jongsakul K. Decrease in Human Immunodeficiency Virus Type 1 Load during Acute Dengue Fever. Clin Infect Dis. 2003;36(8):1067–9.
7. Montoya M, Gresh L, Mercado JC, Williams KL, Vargas MJ, Gutierrez G, et al. Symptomatic Versus Inapparent Outcome in Repeat Dengue Virus Infections Is Influenced by the Time Interval between Infections and Study Year. PLoS Negl Trop Dis. 2013;7(8):1–10.
8. Endy TP, Anderson KB, Nisalak A, Yoon IK, Green S, Rothman AL, et al. Determinants of

inapparent and symptomatic dengue infection in a prospective study of primary school children in Kamphaeng Phet, Thailand. PLoS Negl Trop Dis. 2011;5(3).

9. Torrentes-Carvalho A, Hottz ED, Marinho CF, da Silva JBC, de Oliveira Pinto LM, Fialho LG, et al. Characterization of clinical and immunological features in patients coinfected with dengue virus and HIV. Clin Immunol. 2016;164:95–105.

10. YIN X, ZHONG X, PAN S. CASE REPORT VERTICAL TRANSMISSION OF DENGUE INFECTION: THE FIRST PUTATIVE CASE REPORTED IN CHINA. Rev Inst Med Trop Sao Paulo. 2016;(1):14–7.

11. Maroun SLC, Marliere RCC, Barcellus RC, Barbosa CN, Ramos JRM, Moreira MEL. Case report: Vertical dengue infection. J Pediatr (Rio J). 2008;84(6):556–9.

12. Tan PC, Soe MZ, Lay K, Wang SM, de Sekaran S, Omar SZ. Dengue infection and miscarriage: A prospective case control study. PLoS Negl Trop Dis. 2012 May;6(5).

13. Sondo KA, Ouattara A, Diendéré EA, Diallo I, Zoungrana J, Zémané G, et al. Dengue infection during pregnancy in Burkina Faso: A cross-sectional study. In: BMC Infectious Diseases [Internet]. BioMed Central Ltd.; 2019 [cited 2020 Apr 24]. p. 997. Available from: https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-019-4587-x

14. Sebastião CS, Neto Z, Jesus CS De, Mirandela M, Jandondo D, Couto-Fernandez JC, et al. Genetic diversity and drug resistance of HIV-1 among infected pregnant women newly diagnosed in Luanda , Angola. PLoS One. 2019;1–10.

15. Santiago GA, Vázquez J, Courtney S, Matías KY, Andersen LE, Colón C, et al. Performance of the Trioplex real-time RT-PCR assay for detection of Zika, dengue, and chikungunya viruses. Nat Commun. 2018;9(1).

16. Centers for Disease Control and Prevention. Trioplex Real-time RT-PCR Assay. CDC. 2017;57.

17. Argolo AFLT, Féres VCR, Silveira LA, Oliveira ACM, Pereira LA, Júnior JBS, et al. Prevalence and incidence of dengue virus and antibody placental transfer during late pregnancy in central

Brazil. BMC Infect Dis. 2013;13(1).

18. João EC, Ferreira O da C, Gouvêa MI, Teixeira M de LB, Tanuri A, Higa LM, et al. Pregnant women co-infected with HIV and zika: Outcomes and birth defects in infants according to maternal symptomatology. PLoS One. 2018;13(7):1–12.

19. Yen TY, Trovoada dos Santos M de J, Tseng LF, Chang SF, Cheng CF, Carvalho AV de A, et al. Seroprevalence of antibodies against dengue virus among pregnant women in the Democratic Republic of Sao Tome and Principe. Acta Trop. 2016;155:58–62.

20. Liu L, Huang J, Zhong M, Yuan K, Chen Y. Seroprevalence of Dengue Virus among Pregnant Women in Guangdong, China. Viral Immunol. 2020;33(1):48–53.

21. Stoddard ST, Forshey BM, Morrison AC, Paz-Soldan VA, Vazquez-Prokopec GM, Astete H, et al. House-to-house human movement drives dengue virus transmission. Proc Natl Acad Sci U S A. 2013;110(3):994–9.

22. Leite RC, Souza AI, Castanha PMS, Cordeiro MT, Martelli CT, Ferreira ALG, et al. Dengue infection in pregnancy and transplacental transfer of anti-dengue antibodies in Northeast, Brazil. J Clin Virol. 2014;60(1):16–21.

23. Teixeira MG, Costa MCN, Coelho G, Barreto ML. Recent shift in age pattern of dengue hemorrhagic fever, Brazil. Emerg Infect Dis. 2008;14(10):1663.

24. Rönnberg B, Gustafsson Å, Vapalahti O, Emmerich P, Lundkvist Å, Schmidt-Chanasit J, et al. Compensating for cross-reactions using avidity and computation in a suspension multiplex immunoassay for serotyping of Zika versus other flavivirus infections. Med Microbiol Immunol. 2017;206(5):383–401.

Table 1 Seroprevalence and determinants of DENV infection among HIV-infected pregnant women attending antenatal care in Luanda, Angola, 2018

Patient demographics	No. of pregnant women (%)	Non-infection (IgG-/IgM-)			Past infection (IgG+/IgM-)			Recent infection (IgG-/IgM+ or IgG+/IgM+)		
		No (%)	Yes (%)	p-value	No (%)	Yes (%)	p-value	No (%)	Yes (%)	p-value
Overall	36 (100)	34 (94.4)	2 (5.6)		6 (16.7)	30 (83.3)		32 (88.9)	4 (11.1)	
Age groups										
<25 years	9 (25.0)	9 (100)	0 (0.0)	0.401	3 (33.3)	6 (66.7)	0.121	6 (66.7)	3 (33.3)	0.014*
≥25 years	27 (75.0)	25 (92.6)	2 (7.4)		3 (11.1)	24 (88.9)		26 (96.3)	1 (3.7)	
Place of residence										
Urban area	18 (50.0)	18 (100)	0 (0.0)	0.146	1 (5.6)	17 (94.4)	0.074	17 (94.4)	1 (5.6)	0.289
Rural area	18 (50.0)	16 (88.9)	2 (11.1)		5 (27.8)	13 (72.2)		15 (83.3)	3 (16.7)	
Educational level										
Low	33 (91.7)	31 (93.9)	2 (6.1)	0.661	6 (18.2)	27 (81.8)	0.418	29 (87.9)	4 (12.1)	0.522
High	3 (8.3)	3 (100)	0 (0.0)		0 (0.0)	3 (100)		3 (100)	0 (0.0)	
Occupation										
Unemployed	25 (69.4)	24 (96.0)	1 (4.0)	0.539	5 (20.0)	20 (80.0)	0.418	21 (84.0)	4 (16.0)	0.159
Employed	11 (30.6)	10 (90.9)	1 (9.1)		1 (9.1)	10 (90.9)		11 (100)	0 (0.0)	
Gestational period										
First trimestrer	8 (22.2)	6 (75.0)	2 (25.0)	0.006*	4 (50.0)	4 (50.0)	0.004*	6 (75.0)	2 (25.0)	0.156
Second or third trimestrer	28 (77.8)	28 (100)	0 (0.0)		2 (7.1)	26 (92.9)		26 (92.9)	2 (7.1)	

*The variables were statistically significant for the Chi-square test ($p<0.05$).

335 **Table 2** Determinants of recent DENV infection among HIV-infected pregnant women attending

336 antenatal care in Luanda, Angola, 2018

Patient demographics	Univariate analysis		Multivariate analysis[†]	
	OR (95% CI)	p-value	AOR (95% CI)	p-value
Age groups				
<25 years old	13.0 (1.14-148)	**0.039**	10.0 (0.56-178)	0.117
≥25 years old	1	-	1	-
Place of residence				
Urban area	0.29 (0.03-3.14)	0.311	0.27 (0.02-4.79)	0.374
Rural area	1	-	1	-
Educational level				
Low	1	-	1	-
High	0 (0.0-0.0)	0.999	0 (0.0-0.0)	0.999
Occupation				
Unemployed	1	-	1	-
Employed	0 (0.0-0.0)	0.999	0 (0.0-0.0)	0.999
Gestational period				
First trimester	4.3 (0.50-37.3)	0.182	7.38 (0.43-126)	0.167
Second or third trimester	1	-	1	-

337 Abbreviations: OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio.

338 [†]Adjusted for all the explanatory variables listed.

Manuscrito 3 (Publicado)

Genetic diversity and drug resistance of HIV-1 among infected pregnant women newly diagnosed in Luanda, Angola

PLOS ONE

RESEARCH ARTICLE

Genetic diversity and drug resistance of HIV-1 among infected pregnant women newly diagnosed in Luanda, Angola

Cruz S. Sebastião[1,2,3,4], Zoraima Neto[2], Carlos S. de Jesus[5], Marinela Mirandela[2], Domingos Jandondo[2], José C. Couto-Fernandez[5], Amilcar Tanuri[6], Joana Morais[2,4,7], Miguel Brito[4,8]*

1 NOVA Medical School, Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Lisboa, Portugal, **2** Laboratório de Biologia Molecular, Instituto Nacional de Investigação em Saúde, Luanda, Angola, **3** Instituto Superior de Ciências da Saúde, Universidade Agostinho Neto, Luanda, Angola, **4** Centro de Investigação em Saúde de Angola, Luanda, Angola, **5** Laboratorio de AIDS & Imunologia Molecular, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil, **6** Laboratório de Virologia Molecular, Departamento de Genética, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil, **7** Faculdade de Medicina, Universidade Agostinho Neto, Luanda, Angola, **8** Health and Technology Research Center, Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Lisboa, Portugal

* miguel.brito@estesl.ipl.pt

OPEN ACCESS

Citation: Sebastião CS, Neto Z, de Jesus CS, Mirandela M, Jandondo D, Couto-Fernandez JC, et al. (2019) Genetic diversity and drug resistance of HIV-1 among infected pregnant women newly diagnosed in Luanda, Angola. PLoS ONE 14(11): e0225251. https://doi.org/10.1371/journal.pone.0225251

Editor: Jason Blackard, University of Cincinnati College of Medicine, UNITED STATES

Received: March 6, 2019

Accepted: October 31, 2019

Published: November 26, 2019

Data Availability Statement: The sequences obtained in the study were deposited to GenBank (NCBI) and were assigned the accession numbers MK543512 to MK543545.

Funding: This work was supported by a PhD grant (FCT) (SFRH/BD/135296/2017) to CSS and a grant from project Pró-África CNPq n° 440145/2015-5, Brazil to JCC and AT. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

Monitoring genetic diversity and drug resistance mutations (DRMs) is critical for understanding HIV epidemiology. Here, we report HIV-1 genetic diversity and DRMs in blood samples from 42 HIV-positive pregnant women naive to antiretroviral therapy (ART), in Luanda. The samples were subjected to nested-PCR, followed by sequencing of HIV-1 *pol* gene, targeting the protease and reverse transcriptase fragments. HIV-1 diversity was analyzed using the REGA HIV-1 subtyping tool and DRMs were identified using the Calibrated Population Resistance tool. A total of 34 sequences were obtained. The data revealed wide HIV-1 subtypes heterogeneity, with subtype C (38%, 13/34) the most frequent, followed by the subtypes F1 (18%, 6/34), A1 (9%, 3/34), G (9%, 3/34), D (6%, 2/34) and H (3%, 1/34). In addition, recombinants strains were detected, with CRF02_AG (6%, 2/34) the most frequent, followed by CRF37_cpx, F1/C, A1/G and H/G, all with 3% (1/34). A total of 6/34 (18%) of the sequences presented DRMs. The non-nucleoside reverse transcriptase inhibitors presented 15% (5/34) of resistance. Moreover, 1/34 (3%) sequence presented resistance against both non-nucleoside reverse transcriptase inhibitors and nucleoside reverse transcriptase inhibitors, simultaneously. Despite the small sample size, our results suggest the need to update currently used ART regimens. Surveillance of HIV-1 subtypes and DRMs are necessary to understand HIV epidemiology and to guide modification of ART guidelines in Angola.

Competing interests: The authors have declared that no competing interests exist.

Introduction

The human immunodeficiency virus (HIV) has become a major global public health problem [1], affecting about 36.9 million people in the world [2]. In Angola, a total of 310,000 cases were reported in 2018 [2]. HIV is classified into types (HIV-1 and HIV-2), groups (M, N, O and P), subtypes (A-D, F-H, J and K), sub-subtypes (A1, A2, F1 and F2), circulating recombinant forms (CRFs) and unique recombinant forms (URFs) [3]. HIV-1 is responsible for the vast majority of HIV infections [4]. All subtypes of HIV-1 group M (except B), several CRFs and URFs have been described in Angola [5–11].

Universal access to antiretroviral therapy (ART) has successfully decreased mortality and morbidity associated with HIV [2,12]. The first-line of the ART drugs used in Angola includes the nucleoside reverse transcriptase inhibitors (NRTIs), tenofovir (TDF) and lamivudine (3TC), and a non-nucleoside reverse transcriptase inhibitor (NNRTI), either efavirenz (EFV) or nevirapine (NVP) [13,14]. In addition, zidovudine (AZT) has been used to prevent vertical transmission [13,14].

The emergence of HIV-1 subtypes with drug resistance mutations (DRMs) during pregnancy represents a challenge for the efficacy of ART, especially in low- and middle-income countries [15]. There is a lack of recent data on HIV-1 genetic diversity and prevalence of DRMs in Angola [15,16]. In this study, we investigated the genetic diversity and DRM prevalence in blood samples from HIV-positive pregnant women naive to ART in Luanda, to better understand HIV epidemiology and to allow a timely modification of ART guidelines in Angola.

Materials and methods

Study design and sample collection

A cross-sectional study was carried out at the Lucrecia Paim Maternity clinic, located in Luanda, capital city of Angola, during the months of April to June of 2018. The study involved 1612 pregnant women who were screened for HIV infection using the rapid antibody detection test Determine HIV1/2™ (Alere, Japan) and the Unigold™ HIV (Trinity Biotech, Ireland) during prenatal care. Sociodemographic characteristics and blood samples were collected from HIV-positive pregnant women. The main criterion for inclusion of HIV-positive pregnant women was that they had not been previously exposed to any ART. The blood samples were collected in a tube with EDTA, centrifuged and the plasma was aliquoted and stored at -80˚C. The blood samples preparation was performed at the Molecular Biology Laboratory, of the National Institute for Health Research of Angola (INIS). Following the recommendations of the National Institute of Fighting against AIDS (INLS), the HIV-positive women, were prescribed ART with TDF, 3TC and EFV, and were medicated with AZT until child birth [13,14].

RNA extraction, cDNA synthesis, PCR and sequencing

Total viral RNA was extracted from 140μL of plasma using QIAamp Viral RNA kit (QIAGEN, Germany) following the manufacturer instructions. The cDNA synthesis was carried out using 10μL of the RNA in a final reaction volume of 20μL. The mix contained 25mM DNTP mix, 5X M-MLV buffer, 10mM of dithiothreitol (DTT), 40U of RNase OUT™ (Life Technologies, USA), 0.1mM of MMRTR6 primer (`5′-TTTTACATCATTAGTGTGGG-3′`), and 200U of M-MLV enzyme (Life Technologies, USA) [17].

The obtained cDNA was subjected to a nested-PCR, targeting the protease (PR) and reverse transcriptase (RT) fragments of the HIV-1 *pol* gene, with an expected size of 1302 bp, using the protocol previously described [17]. Successful amplification was checked using a 1%

agarose gel. The amplicons were purified using the NZYGelpure Kit (Nzytech, Portugal), and sequenced using the ABI BigDye Terminator v3.1 reaction kit (Applied Biosystems, USA). For each sample, eight primers were used for the complete sequencing of the PR (nucleotide range: 2253–2549) and the first 335 codons of RT (nucleotide range: 2550–3554), considering the genome of the *HXB2* strain (nucleotide range: 2252–3554) [17]. Sequencing was performed on an ABI 3500 sequencer (Applied Biosystems, USA) at the Molecular Biology Laboratory of the INIS, in Luanda.

HIV-1 subtyping, phylogenetic and resistance mutation analysis

The electropherograms were analyzed using the software RECALL v2.25 [18]. Classification of HIV subtypes was conducted using the REGA HIV-1 subtyping tool v3.0 (http://dbpartners.stanford.edu:8080/RegaSubtyping/stanford-hiv/typingtool/) [19]. The nucleotide sequences obtained were aligned with HIV-1 M-group nucleotide sequences downloaded from NCBI (https://www.ncbi.nlm.nih.gov/nuccore/) and Los Alamos (https://www.hiv.lanl.gov/content/index) databases. The sequences obtained in the study were deposited in GenBank (NCBI) and were assigned the accession numbers MK543512 to MK543545. A phylogenetic tree was inferred using the Neighbor-Joining (NJ) method [20], with Tamura-Nei genetic distances [21]. Clade support was assessed using 1000 bootstrapped replicates [22]. The phylogeny was estimated using MEGA software v7.0 [23], and recombinant viruses were characterized by boot scanning using SimPlot [24].

Drug resistance mutations to HIV-1 were identified using the Calibrated Population Resistance tool (CPR) v8.0 (https://hivdb.stanford.edu/cpr/form/PRRT/) [25]. Additionally, analyses of drug resistance profile were performed using the Stanford genotypic resistance interpretation algorithm (https://hivdb.stanford.edu/hivseq/by-sequences/) [26].

Statistical analysis

Chi-square (X^2) tests were performed to evaluate the association between sociodemographic characteristics and HIV prevalence at 5% statistical significance in the SPSS v25 statistical program (IBM SPSS Statistics, USA).

Ethical considerations

The pregnant women were informed of the study and consented (oral and written) to participation and follow-up until delivery. Consent from parents or guardians of the minors (under 15 years) was also obtained. All pregnant women underwent pre- and post-test counseling individually. The HIV study results were provided to the clinical staff to ensure appropriate patient clinical management. The study protocol was reviewed and approved by the National Ethics Committee of Angola (nr.13/2018), general directorate from Lucrecia Paim Maternity clinic (nr.083/GDG/MLP/2018) and the Ethics Research Committee of the NOVA Medical School/Faculdade de Ciências Médicas—Universidade NOVA de Lisboa (nr.51/2019/CEFCM).

Results

Sociodemographic characteristics

From the 1612 pregnant women tested for HIV, 42 (2.6%) tested positive and not been exposed to any ART previously. All pregnant women were from Luanda province. A total of 25/42 HIV-positive pregnant women were in the age group of 25–34 years, 18/42 had basic and secondary education, respectively, and 19/42 were unemployed. Moreover, a total of 12/42

Table 1. Sociodemographic characteristics of pregnant women tested for HIV in Luanda, Angola, 2018.

Characteristics	Pregnant tested	HIV prevalence		X^2	P-Value
		Negative	Positive		
Age group (years)					
<15	5/1612	4 (80.0%)	1 (20.0%)	11.985	0.017[a]
15–24	610/1612	602 (98.7%)	8 (1.3%)		
25–34	750/1612	725 (96.7%)	25 (3.3%)		
35–44	246/1612	238 (96.7%)	8 (3.3%)		
>44	1/1612	1 (100%)	0 (0.0%)		
Residence (Municipality)					
Luanda	643/1612	623 (96.9%)	20 (3.1%)	7.049	0.424
Viana	408/1612	400 (98.0%)	8 (2.0%)		
Belas	188/1612	181 (96.3%)	7 (3.7%)		
Kilamba Kiaxi	222/1612	216 (97.3%)	6 (2.7%)		
Sambizanga	14/1612	13 (92.9%)	1 (7.1%)		
Cazenga	108/1612	108 (100.0%)	0 (0.0%)		
Cacuaco	26/1612	26 (100.0%)	0 (0.0%)		
Icoli Bengo	3/1612	3 (100.0%)	0 (0.0%)		
Level of education					
Illiterate	72/1612	72 (100.0%)	0 (0.0%)	2.650	0.449
Basic	616/1612	598 (97.1%)	18 (2.9%)		
Secundary	633/1612	615 (97.2%)	18 (2.8%)		
Higher	291/1612	285 (97.9%)	6 (2.1%)		
Occupation					
Unemployed	733/1612	714 (97.4%)	19 (2.6%)	1.124	0.570
Worker	478/1612	463 (96.9%)	15 (3.1%)		
Student	401/1612	393 (98.0%)	8 (2.0%)		
Gestational Age (trimester)					
First	102/1612	93 (91.2%)	9 (8.8%)	33.526	0.000[a]
Second	176/1612	164 (93.2%)	12 (6.8%)		
Third	645/1612	634 (98.3%)	11 (1.7%)		
Parturient[b]	689/1612	679 (98.5%)	10 (1.5%)		

[a] Chi-square (X^2) statistics are significant ($P<0.05$).

[b] Pregnant woman tested for HIV just before labor.

https://doi.org/10.1371/journal.pone.0225251.t001

pregnant women were in the second trimester of gestation, followed by 11/42 in the third trimester and 10/42 pregnant women diagnosed and the sample was obtained just before labor (Table 1).

Genetic diversity analysis

From the 42 plasma samples subjected to nested-PCR, a total of 34 amplicons and respective sequences were obtained. It was not possible to obtain amplicons from the remaining 8 samples, even after repeated PCR attempts using different primers. The genotyping analysis revealed that 28/34 sequences were HIV-1 pure subtypes and 6/34 recombinants strains. From the detected pure subtypes, subtype C (38%, 13/34) was the most frequent HIV-1 subtype, followed by the subtypes F1 (18%, 6/34), A1 (9%, 3/34), G (9%, 3/34), D (6%, 2/34) and H (3%, 1/34). From the recombinants strains detected, CRF02_AG (6%, 2/34), was marginally more frequent, followed by CRF37_cpx, F1/C, A1/G and H/G, all with 3% (1/34) (Fig 1).

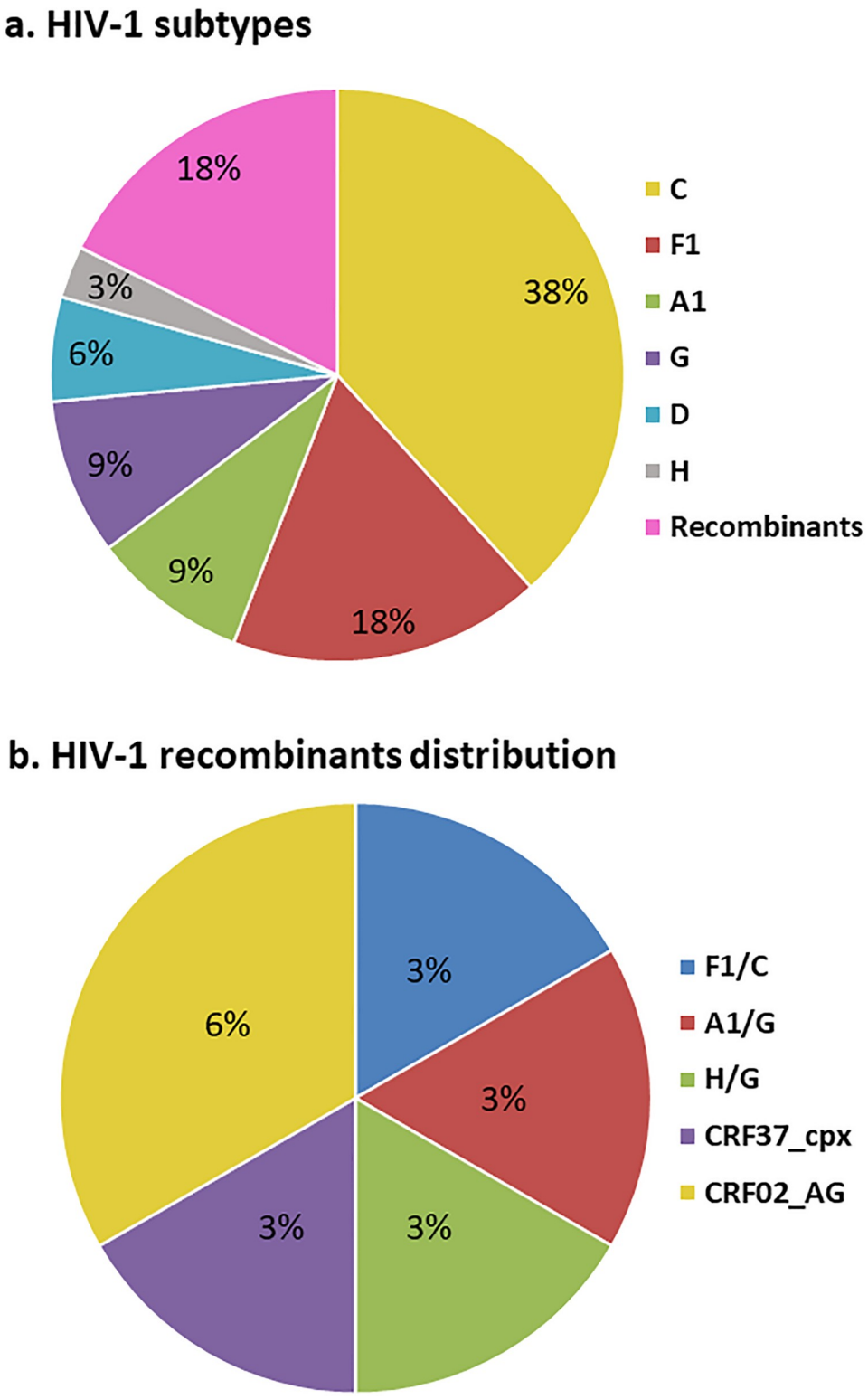


Fig 1. HIV-1 diversity among infected pregnant women in Luanda, Angola, 2018. The analysis involved 34 nucleotide sequences. (A) Frequency of HIV-1 subtypes and recombinants. (B) Recombinants distribution. The subtypes were identified by the REGA v3.0, and recombinants strains by SimPlot.

https://doi.org/10.1371/journal.pone.0225251.g001

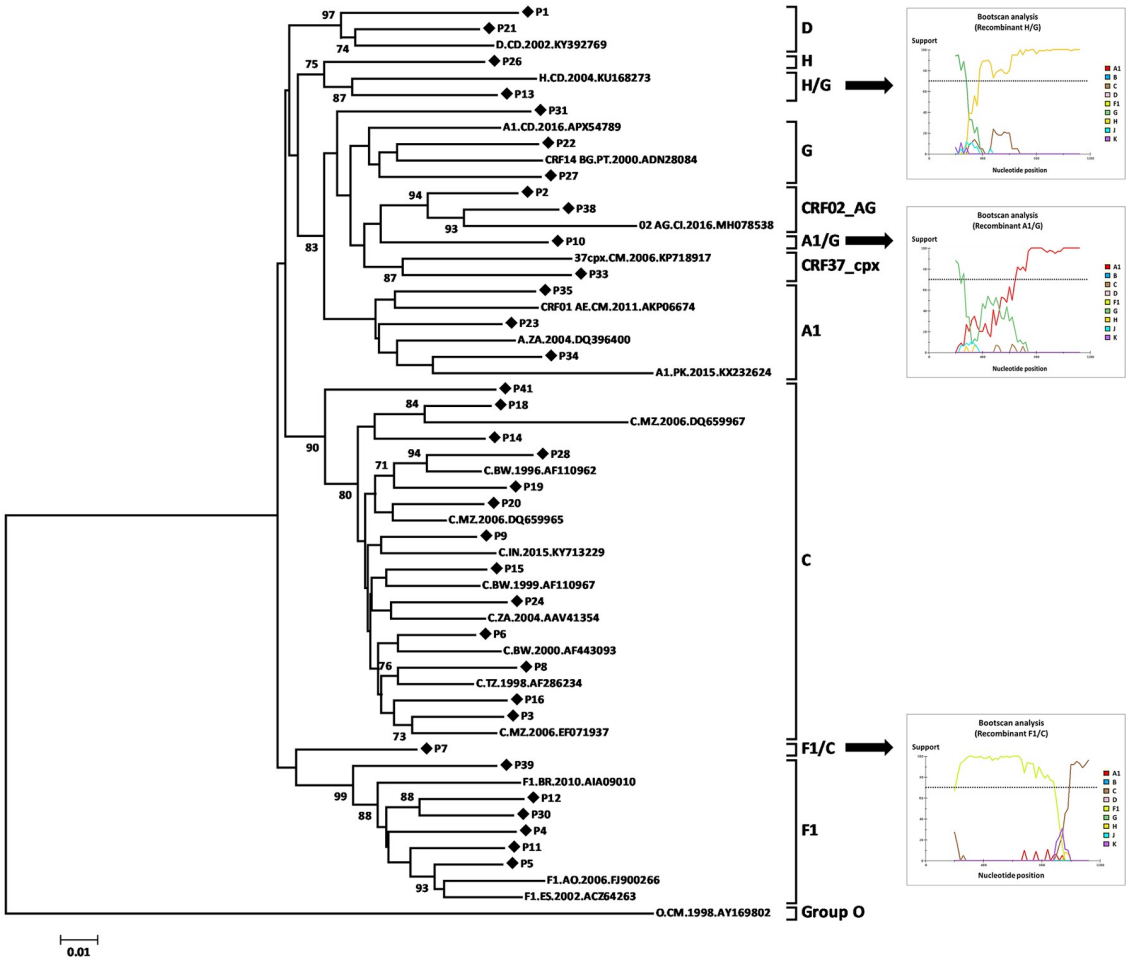


Fig 2. Evolutionary relationships of HIV-1 subtypes among infected pregnant women in Luanda, Angola, 2018. The analysis involved 56 nucleotide sequences. All HIV-1 strains identified in this study were from M-group. The phylogenetic tree was inferred using the NJ method, and Tamura-Nei genetic distances with 1000 bootstrap replicates. Analyses were conducted in MEGA v7.0. Bootstrap values more than 70% and recombinants strains are indicated.

https://doi.org/10.1371/journal.pone.0225251.g002

Genetic distance analysis showed genetic similarity (more than 70%) of HIV-1 subtype C isolates with isolated from Botswana, Mozambique, Tanzania, South Africa and India. The subtype F1 was more similar to isolates from Brazil and Spain. The subtype A1 was most similar to isolates from Cameroon, South Africa, and Pakistan. The subtype G was most similar to isolates from Portugal, and subtype D, subtype H, and recombinant strain H/G to isolates from Democratic Republic of Congo. The recombinants strains A1/G, CRF02_AG, and CRF37_cpx were similar to isolates from Cameroon and Ivory Coast (Fig 2).

Antiretroviral resistance mutations and resistance profile

The sequence analysis showed that 6/34 (18%) of the sequences presented DRMs. Of these, 5/34 (15%) sequences presented resistance to specific NNRTIs. Moreover, 1/34 (3%) sequence presented resistance against both NNRTIs and NRTIs, simultaneously. Mutations at positions K103N (2/34), G190A (2/34), Y181I (1/34), and P225H (1/34) were observed against the class of NNRTIs, whereas mutations at positions M41L, D67N, T69D and T215S were observed against NRTIs. A non-polymorphic protease inhibitor (PI) selected mutation was observed at

Table 2. Drug resistance mutations to NRTI, NNRTI and PI according to the HIV-1 subtypes and resistance profile.

Isolate	HIV-1 subtypes	Drug resistant mutations			Drug resistance profile		
		NRTI	NNRTI	PI	Low	Intermediate	High
P10	A1/G	-	G190A	-	ETR, RPV	EFV	NVP
P12	F1	-	K103N, P225H	-	ETR, RPV	-	EFV, NVP
P26	H	-	G190A	-	ETR, RPV	EFV	NVP
P27	G	M41L, D67N, T69D, T215S	Y181I	-	ABC, TDF	AZT, EFV	ETR, RPV, NVP
P31	G	-	-	I85V	-	-	-
P41	C	-	K103N	-	-	-	EFV, NVP

Antiretroviral drugs: AZT—Zidovudine; EFV—Efavirenz; ETR—Etravirine; NVP—Nevirapine; RPV—Rilpivirine; ABC—Abacavir; TDF—Tenofovir.

https://doi.org/10.1371/journal.pone.0225251.t002

position I85V. High levels of drug resistance were observed for NVP (5/34) and EFV (2/34), intermediate resistance for EFV (3/34) and AZT (1/34) and low resistance for Abacavir (ABC) (1/34) and TDF (1/34). In addition, the susceptibility profile associated to the second generation of NNRTIs was also identified and 1/34 and 3/34 sequence presented high- and low-resistance to Etravirine (ETR) and Rilpivirine (RPV), respectively (Table 2).

Discussion

This study presents an important update on molecular epidemiology of circulating HIV-1 strains in Luanda. The HIV prevalence was 2.6% (42/1612). A significant difference in HIV positivity was observed between pregnant women of different age groups and pregnancy stage ($P<0.05$). On the other hand, residence, level of education and occupation did not show significant differences ($P>0.05$) (Table 1).

Genetic analysis of 34 isolates revealed a wide diversity of HIV-1 strains (Fig 1), similar to that observed in previous studies performed in Angola [5–11]. Though it is hard to prove statistical significance with our small sample size, our study indicates an increase in HIV-1 subtype C, but a slight decrease in subtype F1 in Luanda [5,8]. The genetic similarity of HIV-1 subtype C with isolates from Botswana, Mozambique, Tanzania, South Africa, India and the subtype F1 with isolates from Brazil and Spain (Fig 2), may be attributed to high mobility between countries or to the fact that, after colonial war, and the end of civil war in 2002, thousands of refugees returned to Angola [27].

The RT inhibitors are important components of ART regimen [12–14]. The identification of pre-treatment drug resistance in pregnant women naïve to ART (Table 2), may threaten ART based strategy to HIV control in Luanda [12–14]. The K103N and G190A mutations are associated with EFV and NVP resistance [28]. The Y181I and P225H mutations are often associated with second generation RPV and ETR resistance [29]. The thymidine analogue associated mutations (TAMs) at positions M41L and D67N have the greatest impact on susceptibility of AZT and Stavudine (d4T) [28]. The T69D mutation when present with T215S mutation, is associated with broad resistance to NRTIs [28]. The non-polymorphic PI-selected mutation at position I85V has minimal effects on PIs susceptibility [30,31].

The identification of K103N and G190A mutations, may be attributed to the long use of NNRTIs as part of the first-line ART regimens in Angola [13,14]. Displacement of people to countries where ART is available for a longer period also may help to explain the origin of the HIV-1 subtypes with DRMs in Luanda [27,32].

The reasons for PCR failure of 8/42 samples were not identified. It may be that the high HIV-1 genetic diversity compromised the binding of primers, even though they were targeted at highly conserved regions of the HIV-1. Other studies with more representative sampling

monitoring of the HIV-1 subtypes and DRMs are necessary to guide a timely modification of ART guidelines in Angola. Despite the small sample size, our findings suggest that the Angolan Ministry of Health should prompt consideration of moving to generic integrase strand transfer inhibitors (INSTIs) in the ART regimen in Angola [33].

Conclusions

Our results show a wide HIV-1 subtypes heterogeneity, with subtype C the most frequent. A total of 6/34 (18%) of the sequences presented DRMs. Of these, 15% (5/34) were associated with resistance against NNRTIs. Moreover, 1/34 (3%) sequence presented resistance against both NNRTIs and NRTIs, simultaneously. Our findings suggest the need to update currently used ART regimens. Better understanding is needed of emergence of HIV-1 subtypes and DRMs, to allow a timely modification of ART guidelines in Angola.

Acknowledgments

Thanks to pregnant women who participated in this study. To thank the Postgraduate Science for Development Program (PGCD) and the Foundation for Science and Technology (FCT), by the PhD scholarship awarded to Cruz Sebastião (SFRH/BD/135296/2017), and the project Pró-África (CNPq: 440145/2015-5) for supporting some analysis. Moreover, thanks to INIS, CISA, INLS, FIOCRUZ, NOVA Medical School/Faculdade de Ciências Médicas, ISCISA/Agostinho Neto University, and Lucrecia Paim Maternity clinic, for institutional support. Thanks to Valter Nuaila, Marcio Sitoe, Fredilson Melo and Sarah Hill, for scientific support.

Author Contributions

Conceptualization: Cruz S. Sebastião, Joana Morais, Miguel Brito.

Data curation: Cruz S. Sebastião, Marinela Mirandela, José C. Couto-Fernandez, Joana Morais, Miguel Brito.

Formal analysis: Cruz S. Sebastião, Amilcar Tanuri, Joana Morais, Miguel Brito.

Investigation: Cruz S. Sebastião, Zoraima Neto, Marinela Mirandela, Domingos Jandondo, Joana Morais, Miguel Brito.

Methodology: Cruz S. Sebastião, Zoraima Neto, Carlos S. de Jesus, Marinela Mirandela, Domingos Jandondo, José C. Couto-Fernandez, Amilcar Tanuri, Miguel Brito.

Supervision: Carlos S. de Jesus, Miguel Brito.

Validation: Cruz S. Sebastião, Miguel Brito.

Writing – original draft: Cruz S. Sebastião.

Writing – review & editing: Cruz S. Sebastião, Zoraima Neto, Carlos S. de Jesus, Marinela Mirandela, Domingos Jandondo, José C. Couto-Fernandez, Amilcar Tanuri, Joana Morais, Miguel Brito.

References

1. United Nations. Political Declaration on HIV and AIDS: On the Fast Track to Accelerating the Fight against HIV and to Ending the AIDS Epidemic by 2030. 2016;17020: 1–26.
2. United Nations Joint Programme on HIV/AIDS (UNAIDS). Unaids Data 2018. 2018.
3. Leitner T, Hahn B, Mullins J, Wolinsky S, Foley B, Apetrei C, et al. HIV Sequence Compendium 2015 Editors. Theor Biol Biophys Los Alamos Natl Lab. 2015.

4. Sharp PM, Hahn BH. Origins of HIV and the AIDS pandemic. Cold Spring Harb Perspect Med. 2011; 1: a006841. https://doi.org/10.1101/cshperspect.a006841 PMID: 22229120
5. Afonso JM, Bello G, Monick GL, Sojka M, Morgado MG. HIV-1 Genetic Diversity and Transmitted Drug Resistance Mutations among Patients from the North, Central and South Regions of Angola. PLoS One. 2012; 7: 11. https://doi.org/10.1371/journal.pone.0042996 PMID: 22952625
6. Bártolo I, Rocha C, Bartolomeu J, Gama A, Marcelino R, Fonseca M, et al. Highly divergent subtypes and new recombinant forms prevail in the HIV/AIDS epidemic in Angola: New insights into the origins of the AIDS pandemic. Infect Genet Evol. 2009; 9: 672–682. https://doi.org/10.1016/j.meegid.2008.05.003 PMID: 18562253
7. Bártolo I, Zakovic S, Martin F, Palladino C, Carvalho P, Camacho R, et al. HIV-1 diversity, transmission dynamics and primary drug resistance in Angola. PLoS One. 2014; 9: 1–17. https://doi.org/10.1371/journal.pone.0113626 PMID: 25479241
8. Castelbranco EPAF, da Silva Souza E, Cavalcanti AMS, Martins AN, de Alencar LCA, Tanuri A. Frequency of Primary Resistance to Antiretroviral Drugs and Genetic Variability of HIV-1 Among Infected Pregnant Women Recently Diagnosed in Luanda-Angola. AIDS Res Hum Retroviruses. 2010; 26: 1313–1316. https://doi.org/10.1089/aid.2010.0111 PMID: 20929349
9. Bártolo I, Epalanga M, Bartolomeu J, Fonseca M, Mendes A, Gama A, et al. High Genetic Diversity of Human Immunodeficiency Virus Type 1 in Angola. AIDS Res Hum Retroviruses. 2005; 21: 306–310. https://doi.org/10.1089/aid.2005.21.306 PMID: 15943573
10. Abecasis A, Paraskevis D, Epalanga M, Fonseca M, Burity F. HIV-1 genetic variants circulation in the North of Angola. 2005; 5: 231–237. https://doi.org/10.1016/j.meegid.2004.07.007 PMID: 15737914
11. Garrido C, Zahonero N, Fernándes D, Serrano D, Silva AR, Ferraria N, et al. Subtype variability, virological response and drug resistance assessed on dried blood spots collected from HIV patients on antiretroviral therapy in Angola. J Antimicrob Chemother. 2008; 61: 694–698. https://doi.org/10.1093/jac/dkm515 PMID: 18218644
12. World Health Organization. The use of antiretroviral drugs for treating and preventing hiv infection. World Heal Organ. 2016.
13. National Institute of Fighting against AIDS. Plano Estratégico Nacional Para o Controlo das Infecções de Transmissão Sexual, VIH e SIDA Instituto Nacional de Luta Contra a Sida. 2006.
14. National Institute of Fighting against AIDS. Normas De Tratamento Antirretroviral. 2015; 159.
15. Ssemwanga D, Lihana RW, Ugoji C, Abimiku A, Nkengasong J, Dakum P, et al. Update on HIV-1 acquired and transmitted drug resistance in Africa. AIDS Rev. 2015; 17: 3–20. PMID: 25427100
16. Bbosa N, Kaleebu P, Ssemwanga D. HIV subtype diversity worldwide. Curr Opin HIV AIDS. 2019; 14: 153–160. https://doi.org/10.1097/COH.0000000000000534 PMID: 30882484
17. Delatorre E, Silva-de-Jesus C, Couto-Fernandez JC, Pilotto JH, Morgado MG. High HIV-1 Diversity and Prevalence of Transmitted Drug Resistance Among Antiretroviral-Naive HIV-Infected Pregnant Women from Rio de Janeiro, Brazil. AIDS Res Hum Retroviruses. 2017; 33: 68–73. https://doi.org/10.1089/AID.2016.0159 PMID: 27392995
18. Woods CK, Brumme CJ, Liu TF, Chui CKS, Chu AL, Wynhoven B, et al. Automating HIV drug resistance genotyping with RECall, a freely accessible sequence analysis tool. J Clin Microbiol. 2012; 50: 1936–1942. https://doi.org/10.1128/JCM.06689-11 PMID: 22403431
19. Pineda-peña A, Rodrigues N, Imbrechts S, Libin P, Barroso A, Deforche K, et al. Infection, Genetics and Evolution Automated subtyping of HIV-1 genetic sequences for clinical and surveillance purposes : Performance evaluation of the new REGA version 3 and seven other tools. 2013.
20. Saitou N, Nei M. The Neighbor-joining Method: A New Method for Reconstructing Phylogenetic Trees. 1987; 4: 406–425.
21. Tamura K, Nei M. Estimation of the Number of Nucleotide Substitutions in the Control Region of Mitochondrial DNA in Humans and. 1993; 10.
22. Felsenstein J. Confidence Limits on Phylogenies: an Approach Using the Bootstrap. Int J Org Evol. 1985; 39: 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x PMID: 28561359
23. Kumar S, Stecher G, Tamura K, Medicine E. MEGA7 : Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. 2016; 1–11.
24. Lole KS, Bollinger RC, Paranjape RS, Gadkari D, Kulkarni SS, Novak NG, et al. Full-Length Human Immunodeficiency Virus Type 1 Genomes from Subtype C-Infected Seroconverters in India, with Evidence of Intersubtype Recombination. Am Soc Microbiol. 1999; 73: 152–160.
25. Bennett DE, Camacho RJ, Otelea D, Kuritzkes DR, Fleury H, Kiuchi M, et al. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. PLoS One. 2009; 4. https://doi.org/10.1371/journal.pone.0004724 PMID: 19266092

26. Liu TF, Shafer RW. Web Resources for HIV Type 1 Genotypic-Resistance Test Interpretation. Clin Infect Dis. 2006; 42: 1608–1618. https://doi.org/10.1086/503914 PMID: 16652319

27. Perrin L, Kaiser L, Yerly S. Travel and the spread of HIV-1 genetic variants. Lancet Infect Dis. 2003; 3: 22–27. https://doi.org/10.1016/s1473-3099(03)00484-5 PMID: 12505029

28. Johnson VA, Calvez V, Günthard HF, Paredes R, Pillay D, Shafer R, et al. 2011 Update of the Drug Resistance Mutations in HIV-1. Top Antivir Med. 2011; 156–164. PMID: 22156218

29. Alcaro S, Alteri C, Artese A, Ceccherini-silberstein F, Costa G, Ortuso F, et al. Docking Analysis and Resistance Evaluation of Clinically Relevant Mutations Associated with the HIV-1 Non- nucleoside Reverse Transcriptase Inhibitors Nevirapine, Efavirenz and Etravirine. 2011; 2203–2213. https://doi.org/10.1002/cmdc.201100362 PMID: 21953939

30. Shahriar R, Rhee S, Liu TF, Fessel WJ, Scarsella A, Towner W, et al. Nonpolymorphic Human Immunodeficiency Virus Type 1 Protease and Reverse Transcriptase Treatment-Selected Mutations. 2009; 53: 4869–4878. https://doi.org/10.1128/AAC.00592-09 PMID: 19721070

31. Rhee S, Taylor J, Fessel WJ, Kaufman D, Towner W, Troia P, et al. HIV-1 Protease Mutations and Protease Inhibitor Cross-Resistance. 2010; 54: 4253–4261. https://doi.org/10.1128/AAC.00574-10 PMID: 20660676

32. Van de Vijver D, Wensing AM, Boucher C, others. The epidemiology of transmission of drug resistant HIV-1. Reviews. 2006; 2007: 17–36.

33. Inzaule SC, Hamers RL, Doherty M, Shafer RW, Bertagnolio S, Rinke de Wit TF. Curbing the rise of HIV drug resistance in low-income and middle-income countries: the role of dolutegravir-containing regimens. Lancet Infect Dis. 2019; 19: e246–e252. https://doi.org/10.1016/S1473-3099(18)30710-2 PMID: 30902440

Manuscrito 4 (Publicado)

Clinical and public health implications of HIV-1 genetic diversity and drug resistance mutations in Angola: a systematic review

Contents available at **PubMed**
www.aidsreviews.com

PERMANYER

AIDS Rev. 2021;23:48-56

Clinical and Public Health Implications of HIV-1 Genetic Diversity and Drug Resistance Mutations in Angola: A Systematic Review

*Cruz S. Sebastião[1,2,3,4], Joana Morais[1,2,5], and Miguel Brito[2,6]**

[1]Molecular Biology Laboratory, Instituto Nacional de Investigação em Saúde, Luanda, Angola; [2]Centro de Investigação em Saúde de Angola, Luanda, Angola; [3]Instituto Superior de Ciências da Saúde, Universidade Agostinho Neto, Luanda, Angola; [4]Laboratory of Immunobiology and Pathogenesis of CEDOC, NOVA Medical School, Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Lisboa, Portugal; [5]Faculdade de Medicina, Universidade Agostinho Neto, Luanda, Angola; [6]Health and Technology Research Center, Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Lisboa, Portugal

Abstract

HIV-1 genetic diversity and drug resistance mutations (DRMs) remain a public health concern mainly in low- and middle-income countries. In this review, we estimated the HIV-1 molecular evolution over the past 40 years (1980-2019) in Angola to help guide affordable strategies for HIV-1 epidemic surveillance. We searched for studies written in English or Portuguese on HIV-1 diversity and DRMs carried out in Angola and published between 1980 and 2019. This review yielded eight studies describing a total of 493 samples. No HIV-1 Group N, O, and P were identified, whereas all non-B subtypes from Group M were identified. About 66% of HIV-1 subtypes were pure subtype and 34% recombinant strains. The frequency of recombinant strains increases from 1980 to 2019 (23.6%-41.4%, p<0.001). The subtypes C, F1, CRF02_AG, and the recombinant U/H were the most frequent. One DRM in the PIs was found (I54 M), 22 in the nucleoside reverse transcriptase inhibitors (NRTIs), and 18 in the non-nucleoside reverse transcriptase inhibitors (NNRTIs). The major DRM in the NRTIs was the M184V, whereas the G190A, K103N, and Y181C were the major DRMs in the NNRTIs. Over the past 40 years, the frequency of the DRM M184V (50-64.3%, p=0.363), G190A (17.2-46.2%, p=0.021), and K103N (34.5-42.3%, p=0.551) increased, while the frequency of Y181C (17.2-7.7%, p=0.289) decreased. The current review shows an increase in HIV-1 genetic complexity and DRMs in Angola. Our findings suggest the need to include PIs or integrase strand transfer inhibitors in the first-line antiretroviral therapy regimens in Angola.* (AIDS Rev. 2020;22:48-56)

Corresponding author: Miguel Brito, miguel.brito@estesl.ipl.pt

Key words

HIV-1. Genetic diversity. Drug resistance mutation. Antiretroviral therapy failure. Angola.

***Correspondence to:**
Miguel Brito
E-mail: miguel.brito@estesl.ipl.pt

Received in original form: 21-06-2020
Accepted in final form: 01-09-2020
DOI: 10.24875/AIDSRev.20000057

Introduction

The discovery of human immunodeficiency virus (HIV) as the cause of the AIDS pandemic was one of the major scientific achievements during the last century[1]. At the end of the year 2019, HIV had caused 37.9 million infections and 770,000 died worldwide[2]. An estimated 220,000 infected and 10,000 deaths related to HIV infection have been reported in Angola in the same period[2]. HIV has been divided into two types (type 1 and type 2)[3]. HIV-1 is responsible for the AIDS pandemic and is further divided into groups (M, N, O, and P), subtypes (A-D, F-H, J, and K), sub-subtypes (A1-A4, A6, F1, and F2), circulating recombinant forms (CRFs), and unique recombinant forms (URFs)[3]. In Angola, all non-B subtypes (nBSs) of Group-M have been identified and the pattern of HIV-1 genetic diversity is closer to that described in the border countries as the Republic of Congo, Democratic Republic of Congo (DRC), Zambia and Namibia[4-6].

In recent years, we have witnessed a significant scaleup in access to antiretroviral therapy (ART) in Africa, which has improved the quality of life and survival of HIV-infected patients[7]. The drug classes of nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs) have been used as the backbone of ART mainly in low- and middle-income countries (LMICs)[8,9]. In Angola, the ART guidelines recommend a combination of two NRTIs (tenofovir and lamivudine; tenofovir and emtricitabine; zidovudine and lamivudine; abacavir and lamivudine; and didanosine and lamivudine) and one NNRTI (efavirenz or nevirapine) as part of the first-line ART regimens[10,11]. However, one major challenge associated with universal access to ART is the lack of virological and immunological monitoring as well as the rapid spread of HIV-1 variants with drug resistance mutations (DRMs) in LMICs[12-14].

In the past years, numerous epidemiological studies have addressed the important issue of the epidemiology of transmission of HIV drug resistance (HIVDR)[15]. Furthermore, phylogeographical models supported by a firm statistical basis have been usefully applied to the epidemiological reconstruction of the origin and diffusion of viral infectious diseases in spatial and temporal scales[16]. However, conducting phylogeographic studies able to monitor the spread of HIV-1 subtypes as well as identify the dispersion pathway that drives such changes could be crucial to understand the molecular epidemiology of HIV-1 in Angola. In this systematic review, we aimed to estimate the HIV-1 genetic diversity and the frequency of DRMs over the past 40 years (1980-2019) in Angola. This review allowed us to obtain a stronger picture of the molecular epidemiology of HIV-1, evolutionary and transmission dynamics, subtypes dispersion pathway, and the effectiveness of ART regimens and provides affordable strategies to strengthen the management and surveillance of the HIV-1 epidemic in Angola.

Systematic literature review

We performed a systematic review to identify studies written in English or Portuguese published in peer-reviewed journals based on the following inclusion criteria: first, they must be carried out and published between January 1980 and December 2019. Second, the city/state of sampling data must be performed in regions of Angola. Finally, they must investigate the HIV-1 genetic diversity and DRMs in Angola. The search for articles was performed in May 2020. Keywords related to the subject "HIV-1 genetic diversity and DRMs in Angola" were used to search for available articles on PubMed, Google Scholar, MEDLINE, Cochrane, SCIELO, and Web of Science during the years 1980-2019. We include keywords and search terms as follows: search #1: HIV-1 genetic diversity and drug resistance in Angola. Search #2: HIV-1 genetic diversity in Angola. Search #3: HIV-1 drug resistance in Angola. All relevant original research articles that reported HIV-1 genetic diversity and acquired or transmitted DRMs among drug-naïve and experienced adult, child, or infant patients in Angola were included in the analyses.

Study selection and data extraction

A systematic procedure was used to identify articles relevant to this review. First, all titles and abstracts that addressed other topics than HIV-1 genetic diversity and DRMs in Angola were excluded from the study. Second, all duplicate references were excluded. Finally, all studies that evaluated only HIV-1 genetic diversity and did not evaluate DRMs or vice versa were also excluded from the study. For all articles selected and included in this review, we evaluated the number of participants, sampling strategy, HIV-1 genotyping methods, HIV-1 diversity, and DRMs. No minimum sample size per dataset or sequence length of the *pol* gene was specified and all online HIV-1 subtyping tools or algorithms for

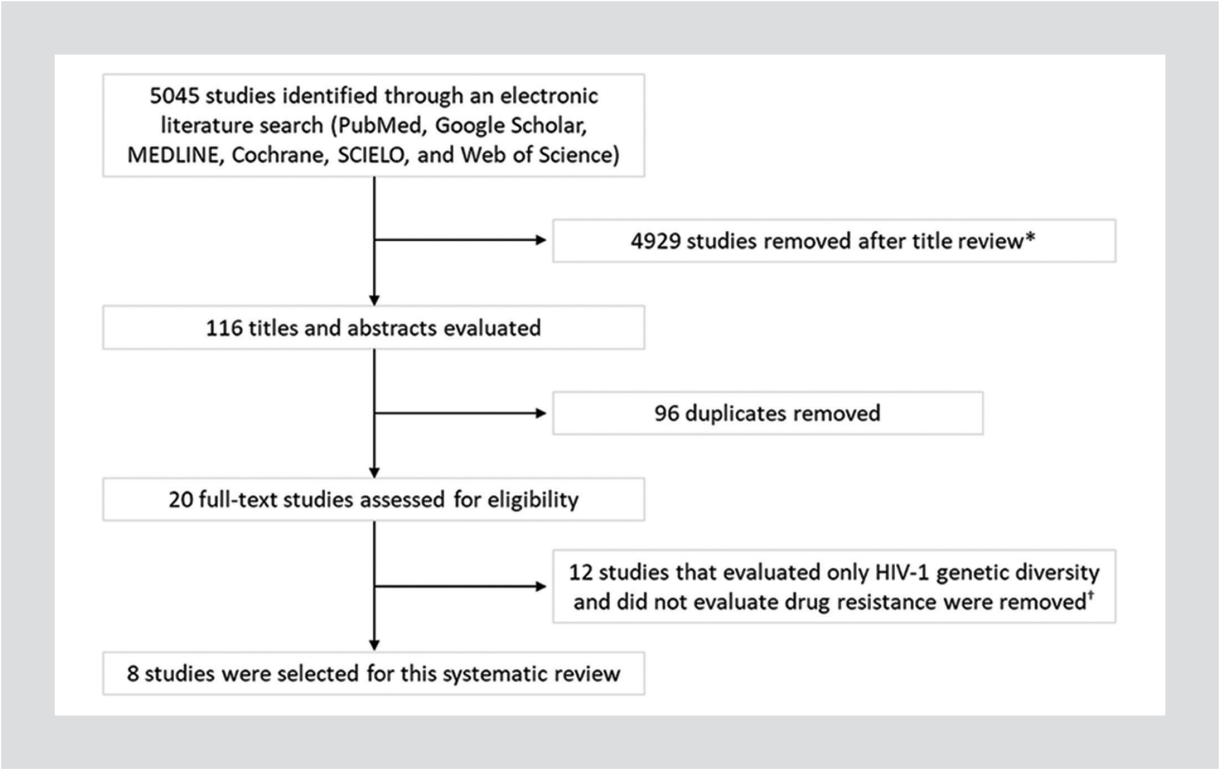


Figure 1. *Flowchart of the selection of studies. All studies were identified through the electronic literature search between 1980 and 2019. The search for articles was performed during May 2020. *For example, antiretroviral therapy for human immunodeficiency virus -2 infection in non-endemic regions. †For example, high genetic diversity of human immunodeficiency virus type 1 in Angola.*

HIVDR evaluation have been accepted. The reviewer (CSS) examined the titles and abstracts, retrieved full-text articles, and assessed articles against eligibility criteria. The reviewer (CSS) extracted the following data from the eligibility articles: city/state of sampling data, the year the studies were conducted and published, population and sampling strategy, HIV-1 genotyping method, genome segment analyzed, results of HIV-1 subtyping, and DRMs. The reviewer (CSS) wrote the original draft. The reviewers (CSS, JM, and MB) performed the review and formal analysis of the data.

Data analysis

The data were analyzed using SPSS version 25 (IBM SPSS Statistics, USA). We evaluated the number of samples designated as HIV-1 subtypes, sub-subtypes, CRFs, and URFs as well as samples presenting any DRM in each data set selected for this review. A Chi-square test was performed to compare proportion and estimate the molecular evolution of HIV-1 and DRMs between the years 1980 and 2019 in Angola. All reported p-values are two tailed and were deemed statistically significant when presented $p<0.05$.

Results

The process of study selection is shown in figure 1. The search found 5045 studies. However, after critical appraisal, a total of eight studies describing a total of 493 samples were found to meet our inclusion criteria and could be included in this review. All selected studies were observational, conducted in Angola, and published in peer-reviewed journals between the years 2000 and 2019. No study was found assessing HIV-1 genetic diversity and DRMs before the year 2000. The summaries of selected studies are shown in table 1.

No HIV-1 Group N, O, and P viruses were identified, whereas all studies identified nBS from Group M. About 66% of HIV-1 subtypes were pure subtype and 34% were recombinant strains. The subtypes A, A1, A2, A3, C, D, F, F1, G, H, J, and K were identified (Fig. 2A). Subtypes C and F1 were the most frequent in almost all studies. Regarding recombinants distribution, CRF02_AG and the recombinant U/H were the most frequent. Furthermore, a vast number of CRFs and URFs have been identified (Fig. 2B). During the years 2000-2019, a significant decrease was observed in the frequency of subtypes A1 (9.9-2.8%, p=0.001), A2

Table 1. HIV-1 molecular epidemiology studies in drug-naïve or treated patients from Angola

Author, year	Location	Population	HIV-1 subtypes	Drug resistance mutations by drug classes
Clemente et al., 2008[20]	Luanda	219 individuals drug-naïve to ART	A, A2, A3, C, D, F, F1, G, H, K, A/C, A/G, A/D, A3/G, CRF01_AE, CRF02_AG, CRF04_cpx, CRF05_DF, CRF11_cpx, G/J, G/U, U/H, and URFs	NRTIs: none NNRTIs: none PIs: none
Garrido et al., 2008[21]	Luanda	294 patients treated	C, F, H, D, G, CRF02_AG, CRF06, CRF01_AE, CRF14_BG, CRF25, CRF19	NRTIs: A62V; D67G; D67N; F116L; K219E; K70R; L74I; M184G; M184V; M41L; T215F; T215I; T215N; T215Y; T69A; T69N; V118D; V118G NNRTIs: A98G; F227V; G190A; K101E; K103N; K103R; V108I; V179D; V179F; Y181C PIs: not evaluated
Ferreira da Silva et al., 2009[27]	Angolan national survey	44 patients	A1, C, D, G, H, and CRF02_AG	NRTIs: M184V; M41L NNRTIs: E138A; V179D; V106I; V90I; Y181H PIs: I54M
Bártolo et al., 2009[22]	Benguela, Cabinda, Cuanza Norte, Cuanza Sul, Luanda, Lunda Norte, Malange and Uíge.	196 individuals drug-naïve to ART	A1, A2, A3, C, D, F1, G, H, J, CRF02_AG, G/H, U/H	NRTIs: D67N; L210W; M184V; M41L; T215F; T215Y NNRTIs: K103N PIs: none
Castelbranco et al., 2010[23]	Luanda	57 individuals drug-naïve to ART	F1, C, A1, D, A/G, G, H, B/D, CRF13_cpx, CRF37_cpx, U/H, and URFs	NRTIs: M184V NNRTIs: G190A PIs: none
Afonso et al., 2012[24]	Central, North and South regions of Angola	101 individuals drug-naïve to ART	C, F1, G, A, D, H, K, CRF02_AG, CRF18_cpx, CRF25_cpx, CRF45_cpx, U/H, and URFs	NRTIs: M184V; M41L; T215F; V75M NNRTIs: G190A; K101E; K103N; M230L; Y106M; Y181C PIs: none
Bártolo et al., 2014[25]	Luanda	139 individuals drug-naïve to ART	HIV-1 subtypes: A, A1, A2, C, D, F1, G, H, J, U/H, U/A, and G/U. HIVDR: 0.7%	NRTIs: none NNRTIs: K103N PIs: none
Sebastião et al., 2019[26]	Luanda	42 individuals drug-naïve to ART	C, F1, A1, G, D, H, CRF02_AG, CRF37_cpx, F1/C, A1/G, and H/G	NRTIs: D67N; M41L; T215S; T69D NNRTIs: G190A; K103N; P225H; Y181I PIs: none

NRTIs: nucleoside reverse transcriptase inhibitors; NNRTIs: non-nucleoside reverse transcriptase inhibitors; PIs: protease inhibitors.

(5.4-1.0%, p=0.004), F (1.5-0%, p=0.038), and H (7.9-3.8%, p=0.050), while a significant increase in frequencies of the recombinant strains (23.6-41.4%, p<0.001) was observed in the same period (Table 2).

A total of 40 DRMs were detected in the reverse transcriptase (RT) fragment (Fig. 3). Of these were reported a total of 22 (55%) mutations conferring resistance to NRTIs (A62V, D67G, D67N, F116L, K219E,

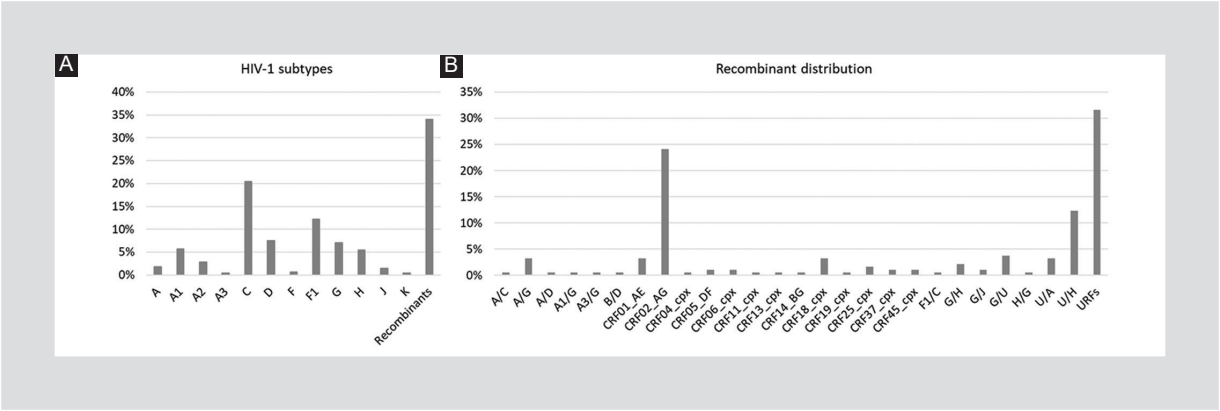


Figure 2. *(**A** and **B**) HIV-1 genetic diversity in Angola, 2000-2019. All reported HIV subtypes belong to the HIV-1 Group M.*

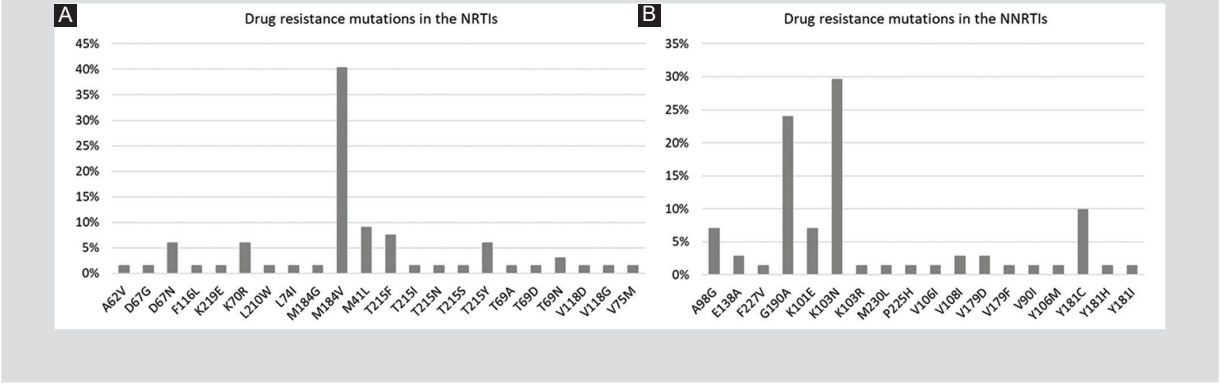


Figure 3. *(**A** and **B**) Prevalence of drug resistance mutations in the reverse transcriptase fragment in Angola, 2000-2019. NRTIs: nucleoside reverse transcriptase inhibitors; NNRTIs: non-nucleoside reverse transcriptase inhibitors.*

K70R, L210W, L74I, M184G, M184V, M41L, T215F, T215I, T215N, T215S, T215Y, T69A, T69D, T69N, V118D, V118G, and V75M) and 18 (45%) mutations conferring resistance to NNRTIs (A98G, E138A, F227V, G190A, K101E, K103N, K103R, M230L, P225H, V106I, V108I, V179D, V179F, V90I, Y106M, Y181C, Y181H, and Y181I). The major DRM in NRTIs was the M184V mutation, whereas the G190A, K103N, and Y181C mutations were the major DRMs in the NNRTIs (Fig. 3). One of the studies reported a major DRM (I54 M) in the protease (PR) fragment between the years 2000 and 2009. Between 2000 and 2019, a significant decrease was observed in the frequency of A98G (17.2-0%, p=0.026), while a significant increase was observed in the frequency of G190A (17.2-46.2%, p=0.021) (Table 2). On the other hand, in the same period, the frequencies of DRM M184V, M41L, T215F, and K103N increased, while the frequencies of D67N, K70R, T215Y, A98G, K101E, and Y181C decreased, although not statistically significant (Table 2).

Discussion

Over the past few years, an increase in the proportion of HIV-1 subtypes has been seen and identified globally[17,18]. The previous studies have shown that subtypes A, C, and CRF02_AG are the most prevalent in Africa, subtype B in Europe and Americas, and CRF01_AE in Asia[18,19]. Because pure subtype B was not identified across all selected studies, our results suggest that it is still absent from Angola. The subtypes C, F1, CRF02_AG, and the recombinant U/H were the most frequent in almost all studies revised. However,

Table 2. HIV-1 molecular evolution and drug resistance mutations in Angola, 1980-2019

Independent variable	n (%)	Years; n (%)				p-value
		1980-1989	1990-1999	2000-2009	2010-2019	
HIV-1 subtypes						
A	9 (1.8)	-	-	1 (0.5)	8 (2.8)	0.064
A1	28 (5.7)	-	-	20 (9.9)	8 (2.8)	0.001*
A2	14 (2.8)	-	-	11 (5.4)	3 (1.0)	0.004*
A3	2 (0.4)	-	-	2 (1.0)	0 (0.0)	0.090
C	101 (20.5)	-	-	42 (20.7)	59 (20.3)	0.926
D	37 (7.5)	-	-	19 (9.4)	18 (6.2)	0.191
F	3 (0.6)	-	-	3 (1.5)	0 (0.0)	0.038*
F1	60 (12.2)	-	-	19 (9.4)	41 (14.1)	0.110
G	35 (7.1)	-	-	16 (7.9)	19 (6.6)	0.571
H	27 (5.5)	-	-	16 (7.9)	11 (3.8)	0.050
J	7 (1.4)	-	-	5 (2.5)	2 (0.7)	0.101
K	2 (0.4)	-	-	1 (0.5)	1 (0.3)	0.799
Recombinant	168 (34.1)	-	-	48 (23.6)	120 (41.4)	<0.001*
HIVDR in NRTIs						
D67N	4 (8.0)	-	-	3 (8.3)	1 (7.1)	0.889
K70R	4 (8.0)	-	-	4 (11.1)	0 (0.0)	0.193
M184V	27 (54.0)	-	-	18 (50.0)	9 (64.3)	0.363
M41L	6 (12.0)	-	-	4 (11.1)	2 (14.3)	0.756
T215F	5 (10.0)	-	-	3 (8.3)	2 (14.3)	0.529
T215Y	4 (8.0)	-	-	4 (11.1)	0 (0.0)	0.193
HIVDR in NNRTIs						
A98G	5 (9.1)	-	-	5 (17.2)	0 (0.0)	0.026*
G190A	17 (30.9)	-	-	5 (17.2)	12 (46.2)	0.021*
K101E	5 (9.1)	-	-	4 (13.8)	1 (3.8)	0.200
K103N	21 (38.2)	-	-	10 (34.5)	11 (42.3)	0.551
Y181C	7 (12.7)	-	-	5 (17.2)	2 (7.7)	0.289

HIVDR: HIV drug resistance; NRTIs: nucleoside reverse transcriptase inhibitors; NNRTIs: non-nucleoside reverse transcriptase inhibitors.
*The variables were statistically significant for the Chi-square test (p<0.05).

the studies revealed that there is no uniform distribution of HIV-1 subtypes in the different regions (South, North, and Center) of Angola. The southern region of Angola has been dominated by subtype C, the northern region by subtype F1, while the central region by a vast number of CRFs and URFs, particularly the CRF02_AG and the new putative CRF, the recombinant U/H[20-27]. However, further analyses with full-length genome sequences of the recombinant U/H are needed to confirm if this represents a novel HIV-1 CRF in Angola. On the other hand, some HIV-1 variants isolated from Angolan patients not allowing clear clustering within the phylogenetic trees when aligned with the global reference strains[20-26]. Therefore, HIV-1 diversity must be carefully analyzed in some regions of Angola[5]. It is worth mentioning that HIV-2 may be also circulating with low endemicity among HIV patients in Angola, which suggests that differential screening for infection or coinfection by HIV-2 should be always excluded at least once in all HIV seroreactive since superinfection with HIV-2 in people infected with HIV-1 or vice versa can occur mainly in endemic regions with both types of HIV[28,29]. Indeed, other studies have shown that due to sociohistorical ties and intense human migration between the 1970s and 1980s, the HIV-2 frequent in Portugal also prevails in a less extent in its former colonies such as Brazil, India, Mozambique, and Angola[30-32]. On the other hand, this intense migratory flow between Portugal and sub-Saharan African countries as Angola may also have contributed to the introduction of HIV-1 nBS strains in Portugal[33]. At present, the global distribution of HIV-1 nBS strains among Portuguese-born individuals has followed a pattern closer to that described in Angola, characterized mainly by the circulation of all HIV-1 nBS and countless recombinant strains[33-35]. The emergence of nBS strains

in Portuguese population might indicate multiple introductions of HIV-1 African variants in Portugal driven mainly by the immigration or international travel of African patients, especially Angolan patients that have presented all the nBS strains, which reflects the close link between Angola and Portugal and the high degree of HIV-1 genetic diversity in Angola[33]. Furthermore, it is worth mentioning that the increase in the frequency of subtype G in Portugal, associated with the intense human migration between Angola and Portugal, could lead to a continuous introduction of subtype G in Angola and worsen the scenario of the HIV-1 genetic diversity in this African country[33-35]. Thus, multiple migration events to countries in the African, European, American, and Asian continent during the colonial war and the subsequent civil war may have had a decisive contribution to the multiple introductions of HIV-1 subtypes as well as the spread of HIV-1 variants in the different regions of Angola[36]. This hypothesis is strongly supported by the fact that the previous studies reported HIV-1 diversity in Angola similar to that observed in border countries such as the Republic of Congo, DRC, Zambia and Namibia[4-6].

The origin of HIV-1 subtype C in Angola remains uncertain and needs further investigations. However, the previous studies suggested the existence of multiple introductions and several autochthonous transmission networks of subtype C, probably resulting from the mobility of the population between Angola and South African border countries over a long period between the late 1970s and the middle 2000s[37]. These findings are consistent with those observed in this systematic review although we observed a slight decrease in the frequency of subtype C (20.7-20.3%, p=0.926) between the years 2000 and 2019 (Table 2). On the other hand, phylogeographic studies showed that the sub-subtype F1 most probably originated in the DRC/Cameroon in the early 1940s, was exported to South America in the early 1950s, and spread to Angola in 1959 from where it was exported to Romania in about 1962 because of the intense political relations between the two countries[38,39]. Over the years 2000-2019, a statistically significant decrease was observed in the frequency of subtype F (1.5-0%, p=0.038), with no significant variation in sub-subtype F1 (9.4-14.1%, p=0.110) in the same period (Table 2). Besides, the evolutionary dynamics of HIV-1 in Angola has been dominated by a significant decrease in the frequencies of sub-subtypes A1 (9.9-2.8%, p=0.001) and A2 (5.4-1.0%, p=0.004), and an increase in the frequency of subtype A (0.5-2.8%, p=0.064) and recombinant strains (23.6-41.4%, p<0.001). However, the increase in circulation of recombinant strains could seriously affect the diagnosis, monitoring, clinical management, as well as the effectiveness of ART regimens used in Angola. It is also worth mentioning that the subtypes J and K have been present in Angola at low levels since at least 1993, suggesting low biological fitness, low transmission efficiency, or unsuccessful introductions in high-risk populations[40]. It was observed the emergence of numerous recombinant strains belonging to subtypes G and H (Fig. 2B), although we observed a decrease in the frequencies of pure subtypes G (7.9-6.6%, p=0.571) and H (7.9-3.8%, p=0.050) (Table 2). However, virological and immunological changes, as well as the response to ART in patients with recombinant strains belonging to subtypes G and H, deserve further investigation.

Similar to our results, the previous studies showed that the NRTIs and NNRTIs are the drug classes with more HIVDR in East, Central, South, and West Africa and that the major DRM in the NRTIs is the M184V mutation, whereas the G190A, K103N, and Y181C mutations are the major in the NNRTIs[14]. In Angola, the NRTIs have been the major cause of HIVDR compared to NNRTIs (Fig. 3). The frequencies of the M184V (50-64.3%, p=0.363), G190A (17.2-46.2%, p=0.021), and K103N (34.5-42.3%, p=0.551) increased, while Y181C (17.2-7.7%, p=0.289) decreased during the years 2000-2019 (Table 2). The unregulated and unmonitored use of antiretroviral drugs bought in the black market or in an abroad country, as well as the displacement of HIV-infected people to countries that ART is accessible for a long time, are the most likely explanations for the emergence of HIVDR in Angola[15,36]. Another explanation is the extensive use of NRTIs and NNRTIs as the backbone of the first-line ART regimens in Angola[10]. The M184V mutation causes resistance to lamivudine, emtricitabine, didanosine, and abacavir, but increases susceptibility to tenofovir, zidovudine, and stavudine[41,42]. The G190A mutation causes resistance to nevirapine and efavirenz, but increased susceptibility to delavirdine[41,42]. The K103N mutation causes resistance to efavirenz and nevirapine, while the Y181C is often associated with efavirenz, nevirapine, etravirine, and rilpivirine[41,42]. In addition, numerous thymidine analog-associated mutations at positions D67N, K70R, K219E, L210W, M41L, and T215Y/F affecting the susceptibility of zidovudine and stavudine were identified in Angolan patients exposed and unexposed to ART over the past 20 years (2000-2019) (Fig. 3A)[41].

The low frequency of DRMs against the PR fragment could be attributed to the fact that PIs are rarely used in the HIV patients in Angola. This limited use of PIs is explained by the fact the first-line ART regimes used in Angola do not include PIs, which indicates that ART regimens containing PIs might be successfully used in the vast majority of Angolan HIV patients experiencing virological or immunological failure[10,11]. Nevertheless, it is worth mentioning that numerous polymorphisms not related to HIVDR have been detected in PR fragment in several Angolan patients, suggesting that some patients could experience virological failure with second-line ART regimens containing PIs[22-26]. Interestingly, some new polymorphisms not previously described in the Stanford database for untreated patients have been identified in the PR (N37I and H69A) and RT (V35Q, Q174V, V245G, D121F, and A272S) fragments in HIV patients from Angola[22]. However, the clinical and public health implications of these polymorphisms in the serological and molecular diagnosis, as well as the effectiveness of ART regimens, should be subject to further studies.

Based on these findings, we showed that a significant number of HIV patients in Angola are on monotherapy with tenofovir or zidovudine and may be experiencing virological or immunological failure. Therefore, we suggest that the Angolan Ministry of Health should prompt consider the possibility of the implement differential HIV-2 screening, implement the routine use of HIV-1 genotyping in all individuals newly diagnosed with HIV, update currently used ART regimens, and include PIs or moving to generic integrase strand transfer inhibitors (INSTIs) in the first-line ART regimen in Angola[43].

Our systematic review had some limitations. Although the coverage of the review was over the past 40 years (1980-2019), few studies on HIV-1 genetic diversity and DRMs carried out in Angola have been found to meet the defined inclusion criteria. The small number of studies as well as the small number of subjects included in this systematic review diminishes the strength of these findings to support the public health prevention program in Angola. Despite these limitations, our findings highlight some important aspects of the molecular epidemiology of HIV-1 that might pose unprecedented clinical and public health implications, particularly for the vaccine development efforts, serological and molecular diagnosis, virological and immunological monitoring, ART, and clinical management of the AIDS pandemic[44,45]. However, further studies are necessary to obtain a stronger picture of the HIV-1 genetic diversity, evolutionary and transmission dynamics, and the impact on the effectiveness of ART regimens used in Angola.

Conclusion

This systematic review has shown that the HIV-1 epidemic increased in genetic complexity over the past 40 years (1980-2019) in Angola. The superinfection with divergent HIV-1 strains has become more common in Angola. The M184V mutation against the NRTIs and G190A, K103N, and Y181C mutations against the NNRTIs has been the major cause of HIVDR which suggests the need to update currently used ART regimens and included the PIs or INSTIs in the first-line ART regimen in Angola. Furthermore, effective and continued surveillance of the evolutionary dynamics of HIV-1 subtypes on an estimated space temporal scale using a phylogeographical reconstruction should be performed in Angola.

References

1. Vahlne A. A historical reflection on the discovery of human retroviruses. Retrovirology. 2009;6:1-9.
2. Unaids. Unaids Data 2019. Unaids; 2019. Available from: https://www.unaids.org/en/resources/documents/2019/2019-UNAIDS-data.
3. Leitner T, Hahn B, Mullins J, Rambaut A, Wolinsky S, Korber B. HIV Sequence Compendium 2015. New Mexico: Theoretical Biology and Biophysics Los Alamos National Laboratory; 2015. Available from: https://www.hiv.lanl.gov/content/sequence/HIV/COMPENDIUM/2015/sequence2015.pdf.
4. Bártolo I, Epalanga M, Bartolomeu J, Fonseca M, Mendes A, Gama A, et al. High genetic diversity of human immunodeficiency virus Type 1 in Angola. AIDS Res Hum Retroviruses. 2005;21:306-10.
5. Bártolo I, Rocha C, Bartolomeu J, Marcelino R, Fonseca M, Mendes A, et al. Highly divergent subtypes and new recombinant forms prevail in the HIV/AIDS epidemic in Angola: new insights into the origins of the AIDS pandemic. Infect Genet Evol. 2009;9:672-82.
6. Pineda-Peña AC, Varanda J, de Sousa JD, Theys K, Bártolo I, Leitner T, et al. On the contribution of Angola to the initial spread of HIV-1. Infect Genet Evol. 2016;46:219-22.
7. United Nations. Political Declaration on HIV and AIDS: on the Fast Track to Accelerating the Fight against HIV and to Ending the AIDS Epidemic by 2030. United States: United Nations; 2016. p. 1-26. Available from: https://www.unaids.org/sites/default/files/media_asset/2016-political-declaration-HIV-AIDS_en.pdf.
8. World Health Organization. Antiretroviral Medicines in Low-and Middle-income Countries. Geneva, Switzerland: World Health Organization; 2013. Available from: https://www.apps.who.int/iris/bitstream/handle/10665/83148/9789241505468_eng.pdf;jsessionid=630804AE53D843E4452120703972EF27?sequence=1.
9. Gilks CF, Crowley S, Ekpini R, Gove S, Perriens J, Souteyrand Y, et al. The WHO public-health approach to antiretroviral treatment against HIV in resource-limited settings. Lancet. 2006;368:505-10.
10. National Institute of Fighting Against AIDS. Normas De Tratamento Antirretroviral. 2015. p. 159. Available from: https://www.aidsfree.usaid.gov/sites/default/files/ao_normastratamentoarv.pdf.
11. National Institute of Fighting Against AIDS. Plano Estratégico Nacional Para o Controlo das Infecções de Transmissão Sexual, VIH e SIDA Instituto Nacional de Luta Contra a Sida; 2006. Available from: http://www.nationalplanningcycles.org/sites/default/files/country_docs/Angola/hiv_plan_angola.pdf.
12. WHO. HIV Drug Resistance Report 2019; 2019. Available from: http://www.scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Who+hiv+drug+resistance+report+2012#5.
13. Wallis CL, Godfrey C, Fitzgibbon JE, Mellors JW. Key factors influencing the emergence of human immunodeficiency virus drug resistance in low-and middle-income countries. J Infect Dis. 2017;216:851-6.
14. Ssemwanga D, Lihana RW, Ugoji C, Abimiku A, Nkengasong J, Dakum P, et al. Update on HIV-1 acquired and transmitted drug resistance in Africa. AIDS Rev. 2015;17:3-20.

15. Van de Vijver D, Wensing AM, Boucher C. The epidemiology of transmission of drug resistant HIV-1. Reviews. 2006;2007:17-36.
16. Lemey P, Rambaut A, Drummond AJ, Suchard MA. Bayesian phylogeography finds its roots. PLoS Comput Biol. 2009;5:1000520.
17. Hemelaar J, Elangovan R, Yun J, Dickson-Tetteh L, Fleminger I, Kirtley S, et al. Global and regional molecular epidemiology of HIV-1, 1990-2015: a systematic review, global survey, and trend analysis. Lancet Infcet Dis. 2018;3099:1-13.
18. Bbosa N, Kaleebu P, Ssemwanga D. HIV subtype diversity worldwide. Curr Opin HIV AIDS. 2019;14:153-60.
19. Sharp PM, Hahn BH. Origins of HIV and the AIDS pandemic. Cold Spring Harb Perspect Med. 2011;1:a006841.
20. Clemente S. Epidemiologia Molecular da Infecção por VIH/SIDA, em Angola. Lisboa: University of Lisbon, Faculdade de Medicina Lisboa; 2008.
21. Garrido C, Zahonero N, Fernándes D, Serrano D, Silva AR, Ferraria N, et al. Subtype variability, virological response and drug resistance assessed on dried blood spots collected from HIV patients on antiretroviral therapy in Angola. J Antimicrob Chemother. 2008;61:694-8.
22. Bártolo I, Rocha C, Bartolomeu J, Gama A, Fonseca M, Mendes A, et al. Antiretroviral drug resistance surveillance among treatment-naive human immunodeficiency virus Type 1-infected individuals in Angola: evidence for low level of transmitted drug resistance. Antimicrob Agents Chemother. 2009;53:3156-8.
23. Castelbranco EP, Da Silva Souza E, Cavalcanti AM, Martins AN, De Alencar LC, Tanuri A. Frequency of primary resistance to antiretroviral drugs and genetic variability of HIV-1 among infected pregnant women recently diagnosed in Luanda-Angola. AIDS Res Hum Retroviruses. 2010;26:1313-6.
24. Afonso JM, Bello G, Guimarães ML, Sojka M, Morgado MG. HIV-1 genetic diversity and transmitted drug resistance mutations among patients from the North, central and South regions of Angola. PLoS One. 2012;7:0042996.
25. Bártolo I, Zakovic S, Martin F, Palladino C, Carvalho P, Camacho R, et al. HIV-1 diversity, transmission dynamics and primary drug resistance in Angola. PLoS One. 2014;130:1-17.
26. Sebastião CS, Neto Z, De Jesus CS, Mirandela M, Jandondo D, Couto-Fernandez JC, et al. Genetic diversity and drug resistance of HIV-1 among infected pregnant women newly diagnosed in Luanda, Angola. PLoS One. 2019;14:1-10.
27. Da Silva RF, Abreu CM, Branco E, Bule E. Evaluation of Primary Resistance in Human Immunodeficiency Virus Type-1 (HIV-1) Circulating in Angola and Mozambique Based on an HIV Drug Resistance Threshold Survey (HIVDR-TS). Fort Myers, Florida, USA: 18th International HIV Drug Resistance Workshop: basic Principles and Clinical Implications; 2009.
28. Rayfield M, de Cock K, Heyward W, Goldstein L, Krebs J, Kwok S, et al. Mixed human immunodeficiency virus (Hiv) infection in an individual: demonstration of both hiv Type 1 and Type 2 proviral sequences by using polymerase chain reaction. J Infect Dis. 1988;158:1170-6.
29. Requena S, Caballero E, Lozano AB, Ríos-Villegas MJ, Benito R, Rojo S, et al. Treatment outcome in dually HIV-1 and HIV-2 coinfected patients living in Spain. AIDS. 2019;33:2167-72.
30. de Mendoza C, Lozano AB, Caballero E, Cabezas T, Ramos JM, Soriano V. Antiretroviral therapy for HIV-2 infection in non-endemic regions. AIDS Rev. 2020;22:44-56.
31. Faria NR, Hodges-Mameletzis I, Silva JC, Rodés B, Erasmus S, Paolucci S, et al. Phylogeographical footprint of colonial history in the global dispersal of human immunodeficiency virus Type 2 Group A. J Gen Virol. 2012;93:889-99.
32. De Mendoza C, Cabezas T, Caballero E, Requena S, Amengual MJ, Peñaranda M, et al. HIV Type 2 epidemic in Spain: challenges and missing opportunities. AIDS. 2017;31:1353-64.
33. Esteves A, Parreira R, Venenno T, Franco M, Piedade J, De Sousa JG, et al. Molecular epidemiology of HIV Type 1 infection in Portugal: high prevalence of non-B subtypes. AIDS Res Hum Retroviruses. 2002;18:313-25.
34. Palma AC, Araújo F, Duque V, Borges F, Paixão MT, Camacho R. Molecular epidemiology and prevalence of drug resistance-associated mutations in newly diagnosed HIV-1 patients in Portugal. Infect Genet Evol. 2007;7:391-8.
35. Carvalho A, Costa P, Triunfante V, Branca F, Rodrigues F, Santos CL, et al. Analysis of a local HIV-1 epidemic in portugal highlights established transmission of Non-B and Non-G subtypes. J Clin Microbiol. 2015;53:1506-14.
36. Perrin L, Kaiser L, Yerly S. Travel and the spread of HIV-1 genetic variants. Lancet Infect Dis. 2003;3:22-7.
37. Afonso JM, Morgado MG, Bello G. Evidence of multiple introductions of HIV-1 subtype C in Angola. Infect Genet Evol. 2012;12:1458-65.
38. Lai A, Ciccozzi M, Franzetti M, Simonetti FR, Bozzi G, Binda F, et al. Local and global spatio-temporal dynamics of HIV-1 subtype F1. J Med Virol. 2014;86:186-92.
39. Bello G, Afonso JM, Morgado MG. Phylodynamics of HIV-1 subtype F1 in Angola, Brazil and Romania. Infect Genet Evol. 2012;12:1079-86.
40. Bartolo I, Calado R, Borrego P, Leitner T, Taveira N. Rare HIV-1 subtype J genomes and a new H/U/CRF02_AG recombinant genome suggests an ancient origin of HIV-1 in Angola. AIDS Res Hum Retroviruses. 2016;32:822-8.
41. Johnson VA, Calvez V, Günthard HF, Paredes R, Pillay D, Shafer R, et al. 2011 Update of the drug resistance mutations in HIV-1. Top Antivir Med. 2011;19:156-64.
42. Wang Y, Xing H, Liao L, Wang Z, Su B, Zhao Q, et al. The development of drug resistance mutations K103N Y181C and G190A in long term nevirapine-containing antiviral therapy. AIDS Res Ther. 2014;11:1-9.
43. Inzaule SC, Hamers RL, Doherty M, Shafer RW, Bertagnolio S, Rinke de Wit TF. Curbing the rise of HIV drug resistance in low-income and middle-income countries: the role of dolutegravir-containing regimens. Lancet Infect Dis. 2019;19:e246-52.
44. Butler I, Pandrea I, Marx P, Apetrei C. HIV genetic diversity: biological and public health consequences. Curr HIV Res. 2007;5:23-45.
45. Santoro MM, Perno CF. HIV-1 genetic variability and clinical implications. ISRN Microbiol. 2013;2013:1-20.

Manuscrito 5 (Publicado)

Factors influencing the HIV drug resistance among pregnant women in Luanda, Angola: Findings from a cross-sectional study

Tropical Medicine and Infectious Disease

MDPI

Communication

Factors Influencing HIV Drug Resistance among Pregnant Women in Luanda, Angola: Findings from a Cross-Sectional Study

Cruz S. Sebastião [1,2,3], Joana Morais [1,2,4] and Miguel Brito [1,5,*]

1 Centro de Investigação em Saúde de Angola, Caxito, Angola; cruzdossantos10@gmail.com (C.S.S.); jmafonso.7@gmail.com (J.M.)
2 Molecular Biology Laboratory, Instituto Nacional de Investigação em Saúde, Luanda, Angola
3 Instituto Superior de Ciências da Saúde, Universidade Agostinho Neto, Luanda, Angola
4 Faculdade de Medicina, Universidade Agostinho Neto, Luanda, Angola
5 Health and Technology Research Center, Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, 1990-096 Lisboa, Portugal
* Correspondence: miguel.brito@estesl.ipl.pt

Abstract: The increase in HIV infection and drug-resistant strains is an important public health concern, especially in resource-limited settings. However, the identification of factors related to the propagation of infectious diseases represents a crucial target offering an opportunity to reduce health care costs as well as deepening the focus on preventing infection in high-risk groups. In this study, we investigate the factors related to drug resistance among HIV-infected pregnant women in Luanda, the capital city of Angola. This was a part of a cross-sectional study conducted with 42 HIV-positive pregnant women. A blood sample was collected, and HIV-1 genotyping was carried out using an in-house method. Multivariate analyses were performed to determine the interaction between sociodemographic characteristics and drug resistance. HIV drug resistance was detected in 44.1% of the studied population. High probabilities of drug resistance were observed for HIV-infected pregnant women living in rural areas (AOR: 2.73; 95% CI: 0.50–14.9) with high educational level (AOR: 6.27; 95% CI: 0.77–51.2) and comorbidities (AOR: 5.47; 95% CI: 0.28–106) and infected with a HIV-1 non-B subtype other than subtype C (AOR: 1.60; 95% CI: 0.25–10.3). The present study reports high HIV drug resistance. Furthermore, older-age, rural areas, high educational levels, unemployed status, having comorbidities, and HIV-1 subtypes were factors related to drug resistance. These factors impact on drug susceptibility and need to be urgently addressed in order to promote health education campaigns able to prevent the spread of drug-resistant HIV strains in Angola.

Keywords: HIV infection; antiretroviral failure; risk factors; pregnant women; Angola

Citation: Sebastião, C.S.; Morais, J.; Brito, M. Factors Influencing HIV Drug Resistance among Pregnant Women in Luanda, Angola: Findings from a Cross-Sectional Study. *Trop. Med. Infect. Dis.* **2021**, *6*, 29. https://doi.org/10.3390/tropicalmed6010029

Received: 4 February 2021
Accepted: 2 March 2021
Published: 5 March 2021

1. Introduction

The human immunodeficiency virus (HIV) remains a global public health problem [1]. In 2019, an estimated 37.9 million people were living with HIV worldwide [2]. Of these, around 220,000 of the infected were living in Angola [2]. The HIV infection is caused by two lentiviruses (HIV-1 and HIV-2) divided into groups (M-P), subtypes (A–D, F–H, J, and K), sub-subtypes (A1, A2, F1, and F2), and circulating recombinant forms (CRFs) [3].

The antiretroviral drugs currently approved to treat HIV infection belong to distinctive classes that act in different phases of the HIV replication as fusion inhibitor, nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), integrase inhibitors, and protease inhibitors (PIs) [4].

Countless factors, including drug-resistant strains, HIV subtypes, viral load monitoring, treatment adherence, comorbidities, and sociodemographic characteristics, have

been associated with the development of drug resistance in HIV-infected pregnant women from low- and middle-income countries (LMICs) [5–10].

The identification of factors related to HIV drug resistance (HIVDR) represents a crucial goal to ensure the effectiveness of antiretroviral treatment (ART). To the best of our knowledge, this is the first study that investigates factors related to the spread of drug resistance mutation (DRMs) in HIV-positive pregnant women in Luanda, the capital of Angola. The information set out in this paper is an extension of our recently published study on HIV-1 diversity and drug resistance in pregnant women [11]. Hence, this study makes it possible to promote health education campaigns and strengthen the ongoing strategies in the control of HIVDR in Angola.

2. Materials and Methods

2.1. Study Design and Setting

This was part of a cross-sectional study carried out by the same research team with 42 HIV-infected pregnant women, recently diagnosed and without prior exposure to ART, between April and June 2018, at the Lucrecia Paim Maternity Hospital in Luanda, the capital of Angola. This is the largest public maternity unit and a reference center for maternal health care, training, and research in Angola. Furthermore, it is a tertiary health institution which provides health care for pregnant women and newborns from all national provinces. The main study inclusion criteria for pregnant women were confirmed diagnosis of HIV infection and no ART exposure. The study protocol was reviewed and approved by the Angolan National Ethics Committee (nr.13/2018) and the General Directorate of Lucrecia Paim Maternity, Luanda, Angola (nr.083/GDG/MLP/2018). The study was verbally explained to the participants and with written informed consent obtained from all participants, or the parents/legal guardians of minors aged under 15, before enrolment in the study.

2.2. Data Collection and Procedure

The research team collaborated with the hospital's teams for data collection. A structured questionnaire served to obtain sociodemographic characteristics, age, place of residence, level of education, occupation, and presence of comorbidities. For each participant, a blood sample was collected using vacutainers, and all prepared serum samples were stored at −80 °C until usage. HIV-1 genotyping test was carried out according to an in-house method as previously described [11]. The HIV nucleotide sequences obtained were submitted to the GenBank (NCBI) database and assigned accession numbers MK543512 to MK543545. HIV subtypes were confirmed by the REGA HIV-1 Subtyping Tool [12]. The identification of HIVDR was assessed with the genotypic resistance interpretation algorithm implemented in the Stanford HIV drug resistance database website (https://hivdb.stanford.edu/hivdb/by-sequences/ accessed in September 2018) [13].

2.3. Statistical Analysis

Statistical analyses were performed by SPSS v25 (IBM SPSS Statistics, Chicago, IL, USA). Categorical variables are presented as frequencies and percentages, and continuous variables as means and standard deviations (SD). Factors related to HIVDR were assessed using a logistic regression model. Univariate and multivariate analysis was performed with all independent variables and an outcome variable. All independent variables were included in the multivariate analysis to ensure that all potentially important variables were maintained. The goodness of fit for the model was verified by the Hosmer–Lemeshow test. The strength and direction of the relationship were determined with an adjusted odds ratio (AOR) and their 95% confidence interval (CI). All reported *p*-values are two-tailed with a level of significance set at 5%.

3. Results

All 42 pregnant women consented to participate in the study, provided the demographic data, and a blood sample for laboratory tests. Analysis of the HIV subtypes and DRMs could only be obtained in 34 out of the 42 samples subjected to HIV-1 genotyping. This was not possible in the remaining samples even after countless repeated attempts applying different conditions and PCR primers. Thus, only data on the 34 pregnant women successfully amplified and genotyped were included in the analyses.

Of the 34 samples genotyped, 15/34 (44.1%) presented DRMs. Out of these 15 pregnant women with mutations, 14/15 (93.3%), 2/15 (13.3%), and 1/15 (6.7%) presented resistance against the NNRTIs, NRTIs, and PIs, respectively. Furthermore, 2/15 (13.3%) samples reported multidrug-resistance, one to NRTIs/NNRTIs and the other to PIs/NNRTIs. The mutations E44ED, M41L, D67N, T69D, and T215S were detected in the NRTIs, whereas the mutations V179E, E138A, V108I, V106I, G190A, P225H, K103N, Y181I, K103Q, and E138G were detected in the NNRTIs. The mutation L33F was the only one detected in the PIs (Table 1).

Table 1. Drug resistance mutations to nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs) according to the HIV-1 subtypes.

HIV-1 Subtypes	HIV-1 Drug Resistance Mutation		
	NRTI	NNRTI	PI
F1	-	V179E	-
F1/C	-	E138A	-
C	-	V108I	-
A1/G	-	V106I; G190A	-
F1	-	P225H; K103N; E138A	-
C	-	E138A	-
C	-	E138A	-
D	E44ED	-	-
H	-	G190A	-
G	M41L; D67N; T69D; T215S	Y181I	-
C	-	E138A	-
CRF37_cpx	-	E138A	-
A1	-	K103Q	L33F
A1	-	E138G	-
C	-	K103N	-

All pregnant women were infected with the non-B subtype (nBS) from HIV-1 group M. Of these, subtype C (n = 13, 38.2%) was the most frequent. Furthermore, subtypes F1, A1, G, D, H, F1/C, A1/G, H/G, CRF02_AG, and CRF37_cpx were found.

The putative factors related to HIVDR are set out in Table 2. The age range was 19 to 42 years. The mean age was 29 ± 6. Most of the pregnant women (n = 28, 82.4%) were aged over 24 years, with high educational levels (n = 21, 61.8%), employed (n = 19, 55.9%), without comorbidities (n = 29, 87.9%), and infected with HIV-1 nBS other than subtype C (n = 21, 61.8%). The analysis returned no significant relationship ($p > 0.05$) and, even so, the multivariate analytical findings demonstrate that the likelihood of developing resistance in pregnant women from rural areas was 2.7 times (95% CI: 0.50–14.9), with a high educational level was 6.3 times (95% CI: 0.77–51.2), with comorbidities was 5.5 times (95% CI: 0.28–106), and with HIV-1 nBS other than subtype C was 1.6 times (95% CI: 0.25–10.3). On the other hand, pregnant women aged under 25 (AOR: 0.28 (95% CI: 0.03–2.91), p = 0.287) and employed pregnant women (AOR: 0.32 (95% CI:0.05–2.10), p = 0.235) were potential protective factors (Table 2).

Table 2. Putative factors related to drug resistance among HIV-positive pregnant women in Luanda, Angola, 2018.

Characteristics	*n* (%)	Resistance to NNRTIs		Resistance to NRTIs		Resistance to PIs		Univariate Analysis		Multivariate Analysis *	
		No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	OR (95% CI)	*p*-Value	AOR (95% CI)	*p*-Value
Overall	34 (100)	20 (58.8)	14 (41.2)	32 (94.1)	2 (5.9)	33 (97.1)	1 (2.9)				
Age groups [a]											
<25 years	6 (17.6)	4 (66.7)	2 (33.3)	6 (100)	0 (0.0)	6 (100)	0 (0.0)	0.58 (0.09–3.68)	0.561	0.28 (0.03–2.91)	0.287
≥25 years	28 (82.4)	16 (57.1)	12 (42.9)	26 (92.9)	2 (7.1)	27 (96.4)	1 (3.6)	1.00	-	1.00	-
Place of residence [b]											
Urban area	17 (50.0)	12 (70.6)	5 (29.4)	16 (94.1)	1 (5.9)	16 (94.1)	1 (5.9)	1.00	-	1.00	-
Rural area	17 (50.0)	8 (47.1)	9 (52.9)	16 (94.1)	1 (5.9)	17 (100)	0 (0.0)	2.06 (0.52–8.18)	0.303	2.73 (0.50–14.9)	0.247
Education [c]											
Low	13 (38.2)	10 (76.9)	3 (23.1)	12 (92.3)	1 (7.7)	13 (100)	0 (0.0)	1.00	-	1.00	-
High	21 (61.8)	10 (47.6)	11 (52.4)	20 (95.2)	1 (4.8)	20 (95.2)	1 (4.8)	2.48 (0.58–10.6)	0.223	6.27 (0.77–51.2)	0.086
Occupation [d]											
Unemployed	15 (44.1)	8 (53.3)	7 (46.7)	14 (93.3)	1 (6.7)	14 (93.3)	1 (6.7)	1.00	-	1.00	-
Employed	19 (55.9)	12 (63.2)	7 (36.8)	18 (94.7)	1 (5.3)	19 (100)	0 (0.0)	0.51 (0.13–2.02)	0.339	0.32 (0.05–2.10)	0.235
Comorbidities [e,#]											
No	29 (87.9)	19 (65.5)	10 (34.5)	27 (93.1)	2 (6.9)	28 (96.6)	1 (3.4)	1.00	-	1.00	-
Yes	4 (12.1)	1 (25.0)	3 (75.0)	4 (100)	0 (0.0)	4 (100)	0 (0.0)	4.91 (0.45–53.3)	0.191	5.47 (0.28–106)	0.261
HIV-1 nBS [f]											
Subtype C	13 (38.2)	8 (61.5)	5 (38.5)	13 (100)	0 (0.0)	13 (100)	0 (0.0)	1.00	-	1.00	-
Others nBS	21 (61.8)	12 (57.1)	9 (42.9)	19 (90.5)	2 (9.5)	20 (95.2)	1 (4.8)	1.46 (0.36–5.95)	0.602	1.60 (0.25–10.3)	0.619

Abbreviations: nBS, non-B subtypes; NNRTIs, non-nucleoside reverse transcriptase inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; PIs, protease inhibitors; OR, odds ratio and their 95% confidence interval (CI); AOR, adjusted odds ratio. Reference class: [a] ≥25 years old; [b] Urban area; [c] Low education; [d] Unemployed; [e] No comorbidities; [f] Subtype C. [#] Missing value = 1. * Adjusted for all the independent variables listed.

4. Discussion

Access to ART is important for reducing mortality and prevents HIV transmission [14,15]. These public health accomplishments were made possible by the widespread administration of standardized ART regimens characterized by an inexpensive fixed-dose combination of NRTIs plus NNRTIs [16]. Although efforts are being made to control HIV, infected people can acquire DRM and can also be infected with drug-resistant strains [17].

In this study, we report a small study with ART-naïve pregnant women in terms of the burden of transmitted DRMs, and to investigate factors related to increasing HIVDR. We found a high (≥15%) drug resistance level according to the WHO [18]. The resulting resistance level was thus higher than those reported in pregnant women from Ghana (<5%) [5], Nigeria (<5%) [7], Malawi (7.6%) [8], and Guinea Bissau (10.4%) [10], while lower than that returned in Tanzania (97%) [9]. The drug resistance level observed in these pregnant women represents a threat to Angola's ART program [19,20].

The identification of the sociodemographic factors of the HIV-affected population is a crucial target for intervention and control of the emergence of DRMs. To the best of our knowledge, this is the first study on factors related to the scatter of HIVDR among pregnant women from Angola. Age, patient residence, educational level, occupation, comorbidities, and HIV-1 subtypes were found to be factors related to the risk of ART failure supported by results recorded by previous studies in LMICs [5–10].

Consistent with our findings, Wilhelmson et al. [10] and Imade et al. [7] reported a high frequency of DRMs in pregnant women aged over 24 years in Guinea Bissau and Nigeria, respectively. Our results are also similar to the findings of Khienprasit et al. [21], which reported a lower likelihood of developing treatment failure in HIV-positive women from urbanized areas, whereas the HIV-positive pregnant women with comorbidities (OR = 1.49 (95% CI = 1.03–2.15), $p = 0.032$) were associated with treatment failure. By contrast, Chagomerana et al. [8] identified how HIV-positive pregnant women with high educational levels were less likely to develop treatment failure, while Wilhelmson et al. observed unfavorable virologic outcomes in pregnant women with low educational level [10].

The protective factor observed in pregnant women aged under 25 years was consistent with studies performed by Khienprasit et al. [21] and Chao et al. [22]. By contrast, Crabtree-Ramírez et al. [23] reported higher odds related to ART failure in young women (OR: 4.7, (95% CI: 1.5–14.4), $p = 0.003$). On the other hand, the protective factor for employed pregnant women was consistent with a study carried out by Bayu et al. [24].

Based on these results, a focus on younger women, employed and from urbanized areas, should be prioritized to ensure better ART outcomes. Additionally, it is urgent to increase the screening for comorbidities and points of care to detect ART failure in rural areas.

Studies showed improved virological outcomes in patients with nBS [25]. However, a widely discussed issue is the impact of ART on nBS once drug design has been performed on subtype B and extended to nBS [26]. Herein, we detect a high likelihood of present DRM in women with other nBS compared to subtype C (AOR: 1.60) (Table 2). For this reason, an update of the algorithms to interpret HIVDR and assess the drugs to which their virus is susceptible should be considered prior to the selection of ART regimens [27].

Our study has limitations. The small sample size limits the conclusions of our study. Besides, this study does not represent the whole population of pregnant women from Luanda or other regions from Angola. The HIV viral load, CD4 count, and other clinical factors were not determined in the enrolled subjects due to a lack of laboratory resources. A low viral load or the high genetic diversity of HIV-1 subtypes circulating in Luanda may explain the failure of the amplification of the viral RNA of the remaining eight samples. Further studies with a larger sample size and participants from other communities in Angola are needed to elucidate the burden of HIVDR in Angolan women. Furthermore,

in settings with a poor roll-out of virological load monitoring, the investigation of the factors related to drug resistance will generate a great impact on the development of more effective public health strategies to control the HIV epidemic in LMICs.

5. Conclusions

Our findings show that HIV drug resistance is a public health burden among pregnant women in Luanda that needs addressing. Older-aged, rural areas, a high educational level, unemployed, comorbidities, and HIV-1 non-B subtypes were factors related to the increase in drug resistance in pregnant women from Luanda. Therefore, if the risk factors related to the spread of HIV drug resistance are not urgently addressed, they may increase hard-to-treat infections and health care costs in Angola.

Supplementary Materials: The following are available online at www.mdpi.com/2414-6366/6/1/29/s1.

Author Contributions: Study design and conceptualization: M.B., J.M., and C.S.S. Sample collection: C.S.S. Laboratory analysis: C.S.S. Data analysis: M.B., C.S.S. Writing of the manuscript: M.B. and C.S.S. Revision of the manuscript: All authors revised the manuscript, read and approved the final version. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Camões, Institute of Cooperation and Language, Portugal; Calouste Gulbenkian Foundation, Portugal; the government of Bengo Province and the Angolan Ministry of Health. CCS holds a grant from the Foundation for Science and Technology (FCT) (SFRH/BD/135296/2017). H&TRC authors gratefully acknowledge FCT/MCTES national support through UIDB/05608/2020 and UIDP/05608/2020.

Institutional Review Board Statement: The study protocol was reviewed and approved by the Angolan National Ethics Committee (nr.13/2018), and the General Directorate of Lucrecia Paim Maternity (nr.083/GDG/MLP/2018).

Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Acknowledgments: We are extremely grateful to all the study participants. We wish to thank the Postgraduate Science for Development Program (PGCD) and the Foundation for Science and Technology (FCT), for the Ph.D. scholarship awarded to CSS (grant number SFRH/BD/135296/2017). Thanks to participant investigators from ISCISA/UAN for their contribution to the collected data. Thanks to investigators of the molecular biology laboratory from INIS and IOC/FIOCRUZ for laboratory support. Thanks to clinical investigators from INLS and Lucrecia Paim Maternity for institutional support.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. UNGASS Political Declaration on HIV and AIDS: On the Fast—Track to Accelerate the Fight against HIV and to End the AIDS Epidemic by 2030. *N. Y.* **2016**, *56350*, 1–27.
2. UNAIDS. *UNAIDS UNAIDS Data 2019 Reference. Joint United Nations Programme on HIV/AIDS*; UNAIDS: Geneva Switzerland, 2019.
3. Foley, B.T.; Leitner, T.K.; Apetrei, C.; Hahn, B.; Mizrachi, I.; Mullins, J.; Rambaut, A.; Wolinsky, S.; Korber, B.T.M. *HIV Sequence Compendium 2015*; National Institutes of Health (NIH): Los Alamos, NM, USA, 2015.
4. World Health Organization (WHO). *Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection. Recommendations for a Public Health Approach (2016)*, 2nd ed.; WHO: Geneva, Switzerland, 2017.
5. Bonney, E.Y.; Addo, N.A.; Ntim, N.A.A.; Addo-Yobo, F.; Bondzie, P.; Aryee, K.E.; Barnor, J.; Brandful, J.; Bekoe, V.; Ohene, S.A.; et al. Low level of transmitted HIV Drug resistance at two HIV care centres in Ghana: A threshold survey. *Ghana Med. J.* **2013**, *47*, 82–86.
6. Wallis, C.L.; Godfrey, C.; Fitzgibbon, J.E.; Mellors, J.W. Key Factors Influencing the Emergence of Human Immunodeficiency Virus Drug Resistance in Low- and Middle-Income Countries. *J. Infect. Dis.* **2017**, *216*, 851–856.
7. Imade, G.E.; Sagay, A.S.; Chaplin, B.; Chebu, P.; Musa, J.; Okpokwu, J.; Hamel, D.J.; Pam, I.C.; Agbaji, O.; Samuels, J.; et al.

Short communication: Transmitted HIV drug resistance in antiretroviral-naive pregnant women in north central Nigeria. *AIDS Res. Hum. Retrovir.* **2014**, *30*, 127–133.
8. Chagomerana, M.B.; Miller, W.C.; Tang, J.H.; Hoffman, I.F.; Harrington, B.J.; DiPrete, B.; Wallie, S.; Jumbe, A.; Limarzi, L.; Hosseinipour, M.C. Prevalence of antiretroviral therapy treatment failure among HIV-infected pregnant women at first antenatal care: PMTCT option b+ in Malawi. *PLoS ONE* **2018**, *13*, 1–12.
9. Ngarina, M.; Kilewo, C.; Karlsson, K.; Aboud, S.; Karlsson, A.; Marrone, G.; Leyna, G.; Ekström, A.M.; Biberfeld, G. Virologic and immunologic failure, drug resistance and mortality during the first 24 months postpartum among HIV-infected women initiated on antiretroviral therapy for life in the Mitra plus Study, Dar es Salaam, Tanzania. *BMC Infect. Dis.* **2015**, *15*, 1–10.
10. Wilhelmson, S.; Månsson, F.; Lindman, J.L.; Biai, A.; Esbjörnsson, J.; Norrgren, H.; Jansson, M.; Medstrand, P. Prevalence of HIV-1 pretreatment drug resistance among treatment naïve pregnant women in Bissau, Guinea Bissau. *PLoS ONE* **2018**, *13*, 1–12.
11. Sebastião, C.S.; Neto, Z.; De Jesus, C.S.; Mirandela, M.; Jandondo, D.; Couto-Fernandez, J.C.; Tanuri, A.; Morais, J.; Brito, M. Genetic diversity and drug resistance of HIV-1 among infected pregnant women newly diagnosed in Luanda, Angola. *PLoS ONE* **2019**, *14*, e0225251.
12. Pineda-Peña, A.-C.; Varanda, J.; Sousa, J.D.d.; Kristof Theys, I.B.; Leitner, T.; Taveira, N.; Vandamme, A.-M.; Abecasis, A.B. On the contribution of Angola to the initial spread of HIV-1 Andrea-Clemencia. *Infect. Genet. Evol.* **2018**, *2016*, 219–222.
13. Liu, T.F.; Shafer, R.W. Web Resources for HIV Type 1 Genotypic-Resistance Test Interpretation. *Clin. Infect. Dis.* **2006**, *42*, 1608–1618.
14. Rodger, A.J.; Cambiano, V.; Bruun, T.; Vernazza, P.; Collins, S.; Van Lunzen, J.; Corbelli, G.M.; Estrada, V.; Geretti, A.M.; Beloukas, A.; et al. Sexual Activity Without Condoms and Risk of HIV Transmission in Serodifferent Couples When the HIV-Positive Partner Is Using Suppressive Antiretroviral Therapy. *JAMA* **2016**, *316*, 171.
15. Rodger, A.J.; Cambiano, V.; Phillips, A.N.; Bruun, T.; Raben, D.; Lundgren, J.; Vernazza, P.; Collins, S.; Degen, O.; Corbelli, G.M.; et al. Risk of HIV transmission through condomless sex in serodifferent gay couples with the HIV-positive partner taking suppressive antiretroviral therapy (PARTNER): Final results of a multicentre, prospective, observational study. *Lancet* **2019**, *393*, 2428–2438.
16. World Health Organization (WHO). *Antiretroviral Medicines in Low-and Middle-Income Countries*; World Health Organization: Geneva, Switzerland, 2013.
17. World Health Organization (WHO). *HIV Drug Resistance Report 2019*; World Health Organization: Geneva, Switzerland, 2019; ISBN 978 92 4 150393 8.
18. WHO. *WH|WHO Global Strategy for the Surveillance and Monitoring of HIV Drug Resistance 2012*; WHO: Geneva, Switzerland, 2014.
19. Instituto Nacional de Luta Contra a Sida. *Plano Estratégico Nacional Para o Controlo das Infecções de Transmissão Sexual, VIH e Sida. Angola*; Instituto Nacional de Luta Contra a Sida: Luanda, Angola, 2006.
20. Instituto Nacional de Luta Contra a Sida. *Normas de Tratamento Antiretroviral, Angola*; Instituto Nacional de Luta Contra a Sida: Luanda, Angola, 2015.
21. Khienprasit, N.; Chaiwarith, R.; Sirisanthana, T.; Supparatpinyo, K. Incidence and risk factors of antiretroviral treatment failure in treatment-naïve HIV-infected patients at Chiang Mai University Hospital, Thailand. *AIDS Res. Ther.* **2011**, *8*, 42.
22. Chao, C.; Tang, B.; Hurley, L.; Silverberg, M.J.; Towner, W.; Preciado, M.; Horberg, M. Risk factors for short-term virologic outcomes among HIV-infected patients undergoing regimen switch of combination antiretroviral therapy. *AIDS Res. Hum. Retrovir.* **2012**, *28*, 1630–1636.
23. Crabtree-Ramírez, B.; Villasís-Keever, A.; Galindo-Fraga, A.; Del Río, C.; Sierra-Madero, J. Effectiveness of highly active antiretroviral therapy (HAART) among HIV-infected patients in Mexico. *AIDS Res. Hum. Retrovir.* **2010**, *26*, 373–378.
24. Bayu, B.; Tariku, A.; Bulti, A.B.; Habitu, Y.A.; Derso, T.; Teshome, D.F. Determinants of virological failure among patients on highly active antiretroviral therapy in University of Gondar Referral Hospital, Northwest Ethiopia: A case-control study. *HIV/AIDS Res. Palliat. Care* **2017**, *9*, 153–159.
25. Scherrer, A.U.; Ledergerber, B.; Von Wyl, V.; Böni, J.; Yerly, S.; Klimkait, T.; Bürgisser, P.; Rauch, A.; Hirschel, B.; Cavassini, M.; et al. Improved virological outcome in white patients infected with HIV-1 non-B subtypes compared to subtype B. *Clin. Infect. Dis.* **2011**, *53*, 1143–1152.
26. Vergne, L.; Snoeck, J.; Aghokeng, A.; Maes, B.; Valea, D.; Delaporte, E.; Vandamme, A.M.; Peeters, M.; Laethem, K. Van Genotypic drug resistance interpretation algorithms display high levels of discordance when applied to non-B strains from HIV-1 naive and treated patients. *FEMS Immunol. Med. Microbiol.* **2006**, *46*, 53–62.
27. Wagner, S.; Kurz, M.; Klimkait, T. Algorithm evolution for drug resistance prediction: Comparison of systems for HIV-1 genotyping. *Antivir. Ther.* **2015**, *20*, 661–665.

Manuscrito 6 (Em revisão)

Antiretroviral activity of novel 3’-Azidothymidine triazole derivatives against wild-type HIV-1 *in vitro*

Antiretroviral activity of novel 3'-Azidothymidine triazole derivatives against wild-type HIV-1 *in vitro*.

(Author list will be included here)

Abstract

The human immunodeficiency virus (HIV) remains a global threat in public health. Current antiretroviral drugs are at risk of being less effective with the emergence of drug-resistant viral variants, especially in low- and middle-income countries. Therefore, there is a crucial need to develop a new generation of more cost-effective drugs with increased efficiency for continued success in antiretroviral treatment. Here we describe the anti-HIV activity and cytotoxicity of eleven novel 3'-Azidothymidine (AZT) triazole derivatives in peripheral blood mononuclear cells (PBMCs). Briefly, a million PBMCs were cultured for seven days prior to infection with GFP-tagged HIV-1 NLENG4-3 strain and treatment with the compounds. The GFP expression was assessed in the viable cell population with flow cytometry and compared to AZT activity. One of the eleven custom AZT derivatives (compound **2**) showed attractive anti-HIV activity with little or no toxicity even when high doses were used. However, any potential clinic benefit of this promising compound will be the subject of future studies.

Keywords: HIV-1 infection; drug research; novel AZT triazole derivatives; cytotoxicity; antiretroviral activity.

Introduction

Human immunodeficiency virus (HIV) affects about 37.9 million people worldwide (1). Currently, antiretroviral (ARV) drugs are the only effective treatment and has successfully decreased the mortality and morbidity associated with HIV infection (2,3). The ARVs can be classified based on their biological target in the HIV life-cycle as: entry inhibitors, nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors, integrase inhibitors and protease inhibitors (4).

NRTIs are the oldest and still build the backbone of current highly active antiretroviral therapy (HAART) against HIV infection (5). NRTIs depend on infected host cells enzymatic processes that result in the formation of respective active triphosphate analogs to exert their ARV activity (6). Despite the structural diversity of NRTIs, active triphosphates analogs are able to effectively mimic the structural contacts of cellular endogenous deoxynucleotide triphosphates (dNTPs) at the active site of HIV reverse transcriptase, allowing efficient incorporation into viral DNA (7,8). All currently approved NRTIs lack a 3'-hydroxyl and compete with endogenous dNTPs for incorporation by HIV reverse transcriptase enzyme that results in obligate chain-termination of viral reverse transcriptase (9). In 1985, 3'-Azidothymidine (AZT) has been identified as the first potent NRTI for the treatment of HIV infection (10). The presence of the 3'-azido group in the AZT prevents the formation of further 5'-3' phosphodiester linkages and consequently, viral DNA synthesis is halted (11).

The effectiveness of HAART has been compromised to some extent by rapid development of multidrug-resistant HIV strains, poor bioavailability and cumulative toxicities (12). Therefore, the need for search for novel anti-HIV agents with better

resistance, pharmacokinetic and toxicity profiles has become urgent mainly with the emergence of drug-resistant viral variants in recent years (13). To this end, alternative strategies of ARVs drug discovery with novel action modes including fighting persistent infection in the viral reservoir have been investigated (14,15). The synthesis and biological evaluation of chemical modification in the 3'-azido group of AZT have been a very active research area by many in the field (16–21). On the other hand, the recent applications of click-chemistry in drug discovery has provides a rapid, efficient and reliable tool for drug discovery for the treatment of viral infectious diseases (22,23).

Although there are countless numbers of compounds with distinct features exhibiting attractive pharmacological properties against HIV infection, there is a crucial need to develop a new generation of more cost-effective drugs with increased efficiency and able to penetrate in the lymphoid tissues. The development of more cost-effective compounds with new features will have a high impact on the costs with treatment of HIV infection, especially in low- and middle-income countries. Herein we report results from a series of novel AZT triazole derivatives obtained by the chemical modification in the 3'-azido group of AZT and screened for anti-HIV activity and cytotoxicity in peripheral blood mononuclear cells (PBMCs), for continued success and further advances in ART.

Materials and Methods

Cells and viruses: PBMCs were separated from buffy coat from healthy donors by density centrifugation on Ficoll-Hypaque (Sigma, St-Luis, MO). Stocks of the GFP-tagged HIV-1 NLENG4-3 strain (HIV-1_{NL4-3}) were obtained through the National Institutes of Health AIDS Reagent Program.

Drug preparation: Eleven novel custom AZT derivatives bearing 1,4- and 1,5-triazoles were synthesized at Instituto de Tecnologia Química e Biológica António Xavier (ITQB) in Lisbon, capital city of Portugal, as described by Alencar *et al.* (24). All custom AZT derivatives (Fig. 1) were dissolved at the concentrations of 10mM and stocked at -20°C.

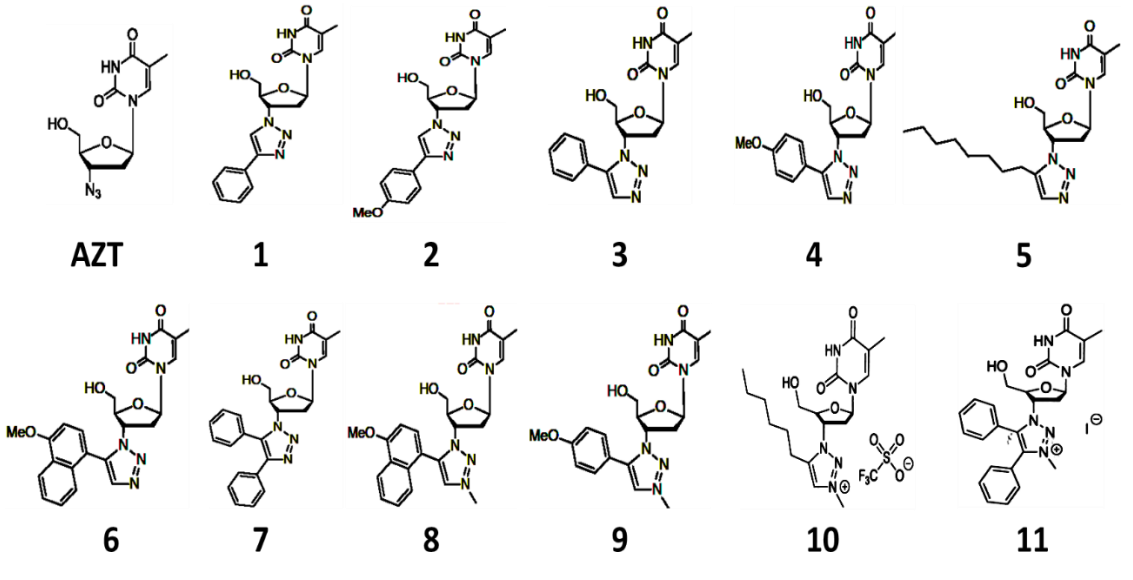


Figure 1. Custom AZT derivatives. AZT represents the gold standard compound, and **1** to **11** represents novel AZT triazole derivatives. Chemical modification in the 3'-azido group of AZT was performed via click-chemistry.

HIV infection: For infection, $1x10^6$ cells/mL were cultured containing human IL-2 (20IU/mL) for 7 days at 37°C in a humidified atmosphere supplemented with 5% CO_2. The culture medium employed was RPMI 1640 (Gibco, life technologies) supplemented with 10% of fetal bovine serum (Biochrom) and 1% of penicillin/streptomycin in the presence of 1% of L-glutamine. The compounds were tested on pretreated and non-pretreated cells. The cells were infected with HIV-1_{NL4-3} in 24-well plates in the presence of Polybrene (8µg/ml). The volume (µL) of the virus suspension was titrated so the control wells exhibited about 80-95% of cell viability. Pretreated cells were harvested 12

hours after culture, centrifuged and resuspended in fresh medium prior to infection. The infected cells were centrifuged at 2500 RPM for 90 minutes at room temperature and maintained in culture for 2 hours. The cells were then washed to remove unattached HIV-1_{NL4-3} and resuspended in 1mL of fresh medium in the presence of the compounds and maintained in culture for 5 days. Initial screening was performed with all compounds at a concentration of 10µM. Dose-response antiviral potency was further assessed for those compounds with promising results in the initial screening. All experiments were conducted in the presence of uninfected cells (cells were treated similarly but were not exposed to the virus), infected cells (cells plus virus), and AZT as control (infected cells plus AZT) (Fig. 2).

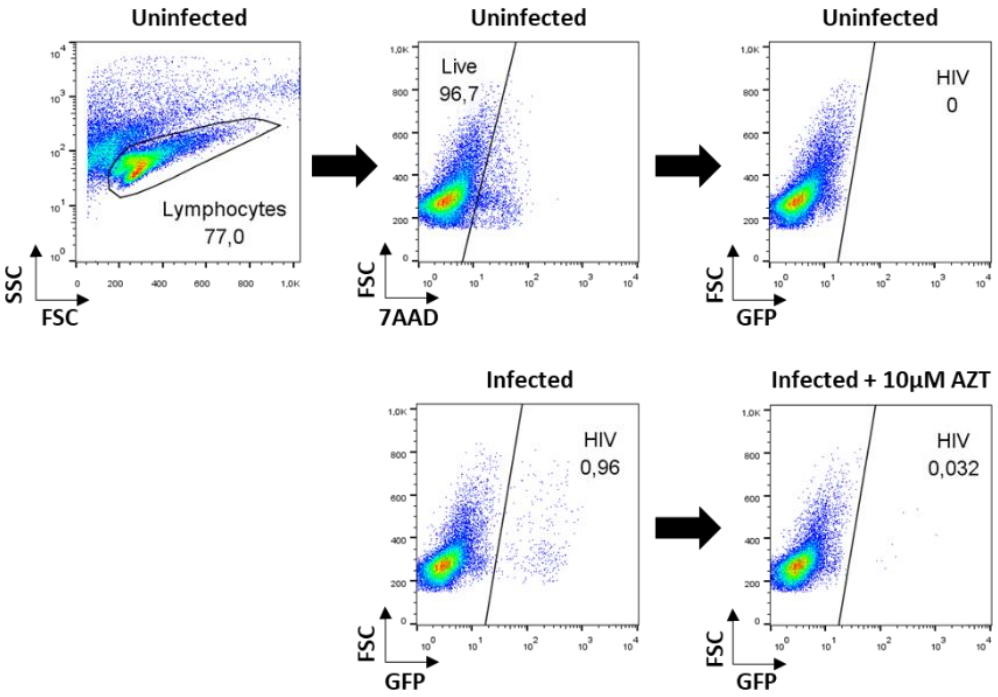


Figure 2: Flow cytometry gating strategy. A million PBMCs were cultured for 7 days prior to infection. Cells were washed and treated with 10µM of compounds, cultured for 5 days, and analyzed with flow cytometry. To exclude cytotoxicity, cells were stained with 7-AAD and GFP expression was measured only in the viable cell population.

The infected cells were stained with 0.2µg/mL of 7-AAD (Sigma-Aldrich) to exclude dead cells and fixed with 1% of paraformaldehyde. The efficiency of HIV-1 replication was assessed for expression of GFP with flow cytometry in FACScan system (BD Biosciences). To exclude other causes that do not involve HIV-1 infection, the gating strategy was evaluated in uninfected cells. Flow cytometry data were analyzed using the software FlowJo v10.

Statistics analyses: Statistical analyses were carried out using the software SPSS v25 (IBM SPSS Statistics, USA) and graphs were plotted on GraphPad prism v7 software (GraphPad Software Inc.). The normality of data distribution was checked using Kolmogorov-Smirnov and Shapiro-Wilk tests or by the values of skewness and kurtosis. Medians and interquartile ranges (IQR) are presented for variables with skewed distributions. Comparisons among treatment groups were analyzed by Mann-Whitney and Kruskal-Wallis tests. All reported p-values are two-tailed with a level of significance of 5%.

Results

General analysis of antiretroviral activity and cytotoxicity of pretreated and non-pretreated cells are summarized in Table 1. The median values of antiretroviral activity in pretreated cells (0.12: 0.06 - 0.18) was lower than non-pretreated cells (0.20: 0.06 - 0.33). On the other hand, both pretreated and non-pretreated cells showed cell viability above 90%. Statistically significant differences were observed in the values of antiretroviral activity and cell viability between pretreated and non-pretreated cells ($p<0.05$).

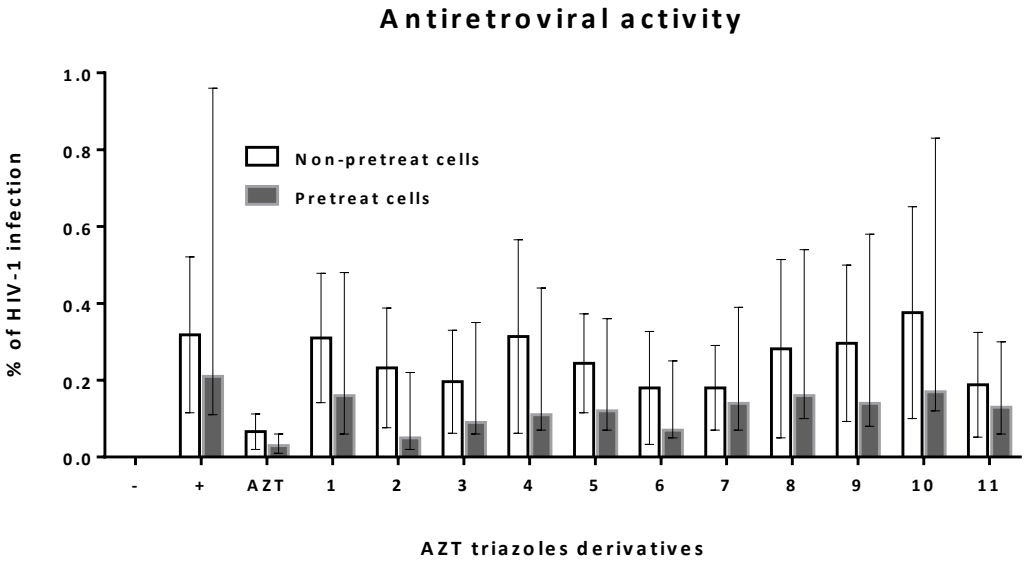
Table 1. Antiretroviral activity and cytotoxicity of the pretreated and non-pretreated cells in the presence of 10µM of the compounds.

Independent variables	Pretreated cells Median (IQR)	Non-pretreated cells Median (IQR)	p-value*
Antiretroviral activity	0.12 (0.06 - 0.18)	0.20 (0.06 - 0.33)	0.048
Cell viability	92.9 (90.9 - 94.1)	93.4 (92.2 - 95.0)	0.011

*Mann-Whitney test

Almost all custom AZT derivatives (91%, 10/11) reduced the HIV-1 infection in the non-pretreated cells, while all compounds reduced the HIV-1 infection in the pretreated cells (Fig. 3A). Cytotoxicity assays were also carried out, and all compounds maintained cell viability above 80% for both methodologies (Fig. 3B).

A



B

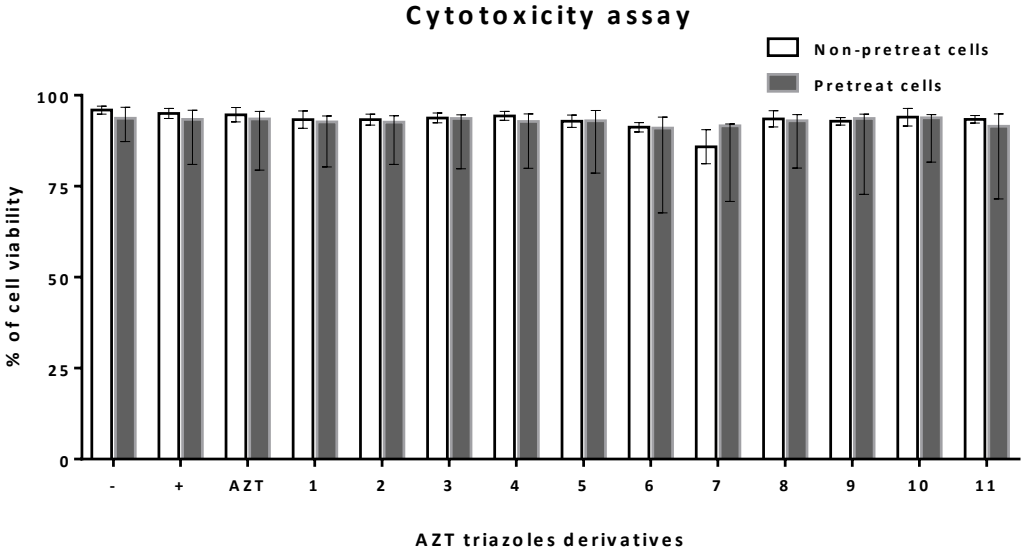
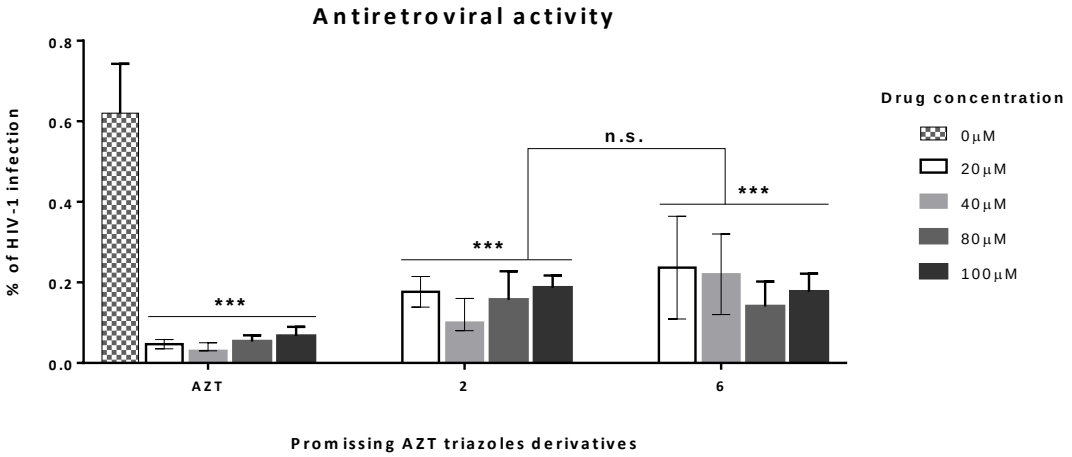


Figure 3. Antiretroviral activity and cytotoxicity of custom AZT derivatives. (A) Antiretroviral activity and (B) cytotoxicity assays. Data shown in A and B represent cumulative results from at least five independent experiments. Error bar: 95% confidence interval (CI). Kruskal-Wallis test showed statistically significant differences in the values of antiretroviral activity and cytotoxicity ($p<0.001$). Abbreviation: (-) indicates uninfected cells; (+) indicates infected cells; (AZT) indicates gold standard compound; (1 to 11) indicates custom AZT derivatives.

Compounds **2** (1,4-triazole) and **6** (1,5-triazole) exhibited attractive anti-HIV activity at a concentration of 10µM with little or no toxicity in the pretreated cells (Fig. 3). Dose-response antiviral potency was evaluated on these compounds (compounds **2** and **6**) using pretreated cells. The promising compounds reduced the HIV-1 infection more than 50% at all indicated dosages (20-100µM) (Fig. 4A). Kruskal-Wallis test showed statistically significant differences between antiviral activity ($p<0.001$), while no significant differences were observed for cytotoxicity assays ($p=0.128$), compared to AZT.

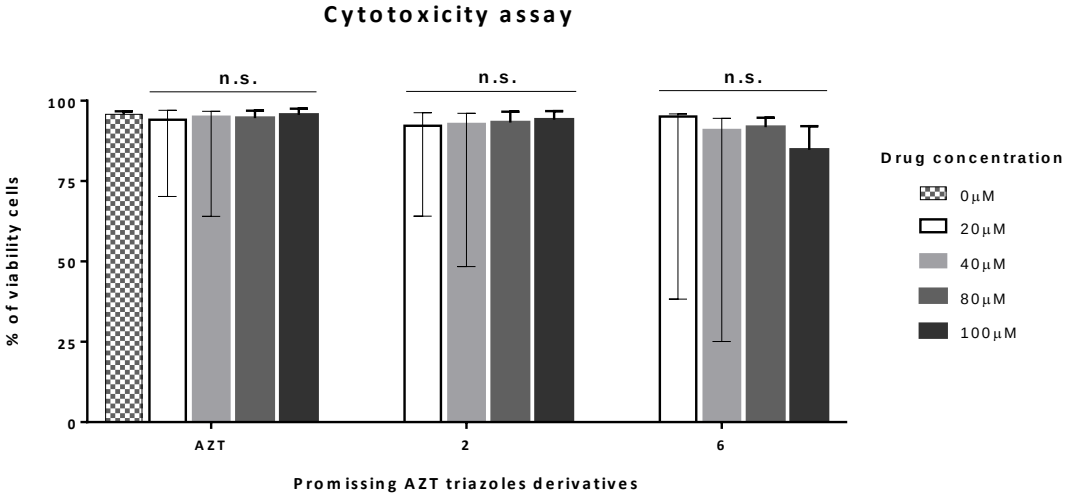
146

147 **A**



148

149 **B**



150

151 **Figure 4. Dose-response of promising AZT triazole derivatives.** Pretreated cells were washed and
152 cultured for 5 days after infection in the absence and presence of various concentrations of compounds.
153 Similarly analyzed with flow cytometry was assessed as described in Fig. 2. (A) Antiretroviral activity and
154 (B) cytotoxicity assay. Data shown in A and B represent cumulative results from at least three independent
155 experiments. Error bar: 95% CI. Abbreviation: (***) indicates $p<0.001$ (Kruskal-Wallis test); (n.s.) indicates
156 not significant.

Discussion

Although the universal access to ART have been successful in controlling HIV infection (2,3), the development of more cost-effective drugs with increased efficiency, reduced side effects and little or no cytotoxicity so that they can be used in long-term therapy remains necessary. Therefore, pharmacomodulation has become central to drug discovery for viral infectious diseases (13). Due to the efforts of numerous researchers in the field of drug design, countless numbers of compounds with chemical modification in the 3'-azido group of AZT have been synthesized and tested as experimental drugs to inhibit HIV replication (16–20). Therefore, compounds **1** to **11** were synthesized and evaluated in pretreated and non-pretreated cells to assess their ability to pre-exposure prophylaxis (PrEP) and post-exposure prophylaxis (PEP), respectively.

The able to decrease HIV-1 reverse transcriptase activity was observed in almost all compounds (Fig. 3A). However, the most attractive reduction of HIV-1 infection was observed in the pretreated cells (Table 1), indicating that these compounds may play an important role in PrEP. On the other hand, all compounds showed a substantial level of viable cells (85-94%) at a concentration of 10µM for both methodological approach (Fig. 3B). In addition, when added in the absence of HIV-1 infection, no custom AZT derivatives exerted any cytotoxic effect (results not shown).

Of the eleven custom AZT derivatives, compounds **2** and **6** showed promising results at a concentration of 10µM. Furthermore, dose-response evaluation revealed that the compound **2** has attractive anti-HIV activity with little or no toxicity even when high doses were used. On the other hand, the inhibition of HIV-1 replication by compound **6**

likely resulted from direct killing of HIV-infected cells with increasing drug concentration (Fig. 4). The effective dose (ED) of compound **2** was observed with 40µM (82% inhibition of HIV-1 replication), while the ED of compound **6** was 80µM (78% inhibition of HIV-1 replication) (Fig. 4A). Interestingly, the ED of AZT was similar to that observed for compound **2**. This finding shows that compound **2** may be an alternative to AZT as PrEP or PEP to block the acquisition of HIV-1 infection.

As proposed by Sirivolu *et al*. (21), substitution patterns and introduction of a bulk aromatic group into C4 or C5 in the 3'-azido group are the main features for AZT derivatives to exhibit anti-HIV activity. However, among the wide range of chemical modification performed in the 3'-azido group of AZT, only compounds **2** exhibited attractive pharmacological properties. Interestingly, compound **2** showed better anti-HIV activity compared to synthesized 1,5-regioisomer (compound **4**). On the other hand, contrary results were observed between compounds **1** and **3** (1,5-regioisomer). Discernible antiviral activities between AZT-derived regioisomers have also been observed in previous studies (21), suggesting that minor modifications to the AZT structure may lead to loss of antiviral activity and increase toxicity.

Additionally, we wanted to ascertain whether the presence of the bulky aromatic group simultaneously on C4 and C5 (compounds **7** and **11**) or a long hydrocarbon chain length at C5 (compounds **5** and **10**) in the 3'-azido group would have attractive pharmacological properties. Our results showed that a long chain length of 6-8 carbons in the C5 of the 3'-azido group of AZT is not essential to inhibit HIV-1 replication. On the other hand, the presence of the bulky aromatic group simultaneously on C4 and C5 reduced the anti-HIV activity and increase cytotoxicity levels.

It is worth stressing that although compound **2** has attractive antiretroviral activity *in vitro* does not ensure that it will be clinically useful in the treatment of viral infectious diseases, since metabolic features, bioavailability, and other factors could negate the clinical utility of a promising compound (6,9). However, further characterization of the antiviral profile and intracellular metabolism using AZT-resistant HIV-1 strains or to other classes of ARVs is necessary to better understand the biological activity of this promising compound.

The resulting inactivity of AZT derivative screened (compounds **1**, **3-5**, **7-11**) as antiviral agents may be ascribed to the volume of the aromatic group or substitution patterns in the 3'-azido group of AZT. On the other hand, NRTIs require successive phosphorylation steps by host cell kinases and phosphotransferase that result in formation of their active triphosphates analogs (6,7). Therefore, inefficient intracellular phosphorylation and little substrate affinity for the viral polymerases may help explain the markedly reduced anti-HIV activity in the remaining compounds (6–9). Based on these promising *in vitro* data, further exploration of the intracellular pharmacokinetic profile of the triphosphate with chemical modification in the 3'-azido group of AZT and their effects on antiretroviral activity and cytotoxicity remain necessary.

Conclusions

Our findings show that there are considerable restrictions on chemical modification in the 3'-azido group of AZT. Among a wide range of subsequently substitutions performed in this position, only compound **2** showed attractive anti-HIV activity with little or no toxicity even when high doses were used. Thus, compound **2** is a novel AZT triazole

derivative with high potential for further investigation in the treatment of HIV-1 infection or pre-exposure and post-exposure prophylaxis.

Acknowledgments

References

1. Unaids. Unaids Data 2019. Unaids [Internet]. 2019; Available from: https://www.unaids.org/en/resources/documents/2019/2019-UNAIDS-data

2. Broder S. The development of antiretroviral therapy and its impact on the HIV-1/AIDS pandemic. Antiviral Res. 2010;85(1):1–18.

3. United Nations. Political Declaration on HIV and AIDS: On the Fast Track to Accelerating the Fight against HIV and to Ending the AIDS Epidemic by 2030. 2016;17020(June):1–26. Available from: https://www.unaids.org/sites/default/files/media_asset/2016-political-declaration-HIV-AIDS_en.pdf

4. World Health Organization. The use of antiretroviral drugs for treating and preventing hiv infection. World Heal Organ [Internet]. 2016; Available from: https://apps.who.int/iris/bitstream/handle/10665/208825/9789241549684_eng.pdf;jsessionid=5D8161935B8E5B01686201A615320C81?sequence=1

5. Cihlar T, Ray AS. Nucleoside and nucleotide HIV reverse transcriptase inhibitors:

25 years after zidovudine. Antiviral Res. 2010;85(1):39–58.

6. Stein DS, Moore KHP. Phosphorylation of nucleoside analog antiretrovirals: A review for clinicians. Pharmacotherapy. 2001;21(1):11–34.

7. Sarafianos SG, Marchand B, Das K, Himmel DM, Parniak MA, Hughes SH, et al. Structure and Function of HIV-1 Reverse Transcriptase: Molecular Mechanisms of Polymerization and Inhibition. J Mol Biol [Internet]. 2009;385(3):693–713. Available from: http://dx.doi.org/10.1016/j.jmb.2008.10.071

8. Katherine L. Seley-Radtke, Yates MK. The evolution of nucleoside analogue antivirals: A review for chemists and non-chemists. Part 1: Early structural modifications to the nucleoside scaffold. HHS Public Access. 2018;154(1):66–86.

9. Anderson PL, Kakuda TN, Lichtenstein KA. The Cellular Pharmacology of Nucleoside- and Nucleotide-Analogue Reverse-Transcriptase Inhibitors and Its Relationship to Clinical Toxicities. Clin Infect Dis. 2004;38(5):743–53.

10. Mitsuya H, Weinhold KJ, Furman PA, St Clair MH, Lehrman SN, Gallo RC, et al. 3'-Azido-3'-deoxythymidine (BW A509U): An antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus in vitro. Med Sci. 1985;82(20):7096–100.

11. Veal GJ, Back DJ. Metabolism of zidovudine. Gen Pharmacol. 1995;26(7):1469–75.

12. World Health Organization (WHO). HIV Drug Resistance Report 2019 [Internet]. 2019. 27 p. Available from:

http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Who+hiv+drug+resistance+report+2012#5

13. Jochmans D. Novel HIV-1 reverse transcriptase inhibitors. Virus Res. 2008;134(1–2):171–85.

14. Zhan P, Pannecouque C, De Clercq E, Liu X. Anti-HIV Drug Discovery and Development: Current Innovations and Future Trends. J Med Chem. 2016;59(7):2849–78.

15. Richman DD, Margolis DM, Delaney M, Greene WC, Hazuda D, Pomerantz RJ. The Challenge of Finding a Cure for HIV Infection. Science (80-). 2009;323(March):1304–7.

16. Roy V, Obikhod A, Zhang H-W, Coats SJ, Herman BD, Sluis-Cremer N, et al. Synthesis and Anti-HIV Evaluation of 3'- Triazolo Nucleosides. Nucleosides, Nucleotides and Nucleic Acids. 2011;(September):37–41.

17. Lazrek HB, Taourirte M, Oulih T, Barascut JL, Imbach JL, Pannecouque C, et al. Synthesis and anti-HIV activity of new modified 1,2,3-triazole acyclonucleosides. Nucleosides, Nucleotides and Nucleic Acids. 2001;20(12):1949–60.

18. Hirota K, Hosono H, Kitade Y, Maki Y, Chu CK, Schinazi RF, et al. Synthesis and Anti-Human Immunodeficiency Virus (HIV-1) Activity of 3'-deoxy-3'-(triazol-1-yl)thymidines and 2',3'-dideoxy-3'-(triazol-1-yl)uridines and Inhibition of Reverse Transcriptase by Their 5'-triphosphates. Chem Pharm Bull. 1986;34(1):430–3.

19. Wigerinck P, Aerschot A Van, Janssen G, Claes P, Balzarini J, Clercq E De, et al.

Synthesis and Antiviral Activity of 3’-Heterocyclic Substituted 3’-Deoxythymidines. J Med Chem. 1990;868–73.

20. Hong-wang Z, Coats SJ, Bondada L, Amblard F, Detorio M, Asif G, et al. Synthesis and evaluation of 3’-azido-2’,3’-dideoxypurine nucleosides as inhibitors of human immunodeficiency virus. Bioorg Med Chem Lett [Internet]. 2011;23(1):1–7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf

21. Sirivolu VR, Vernekar SK V., Ilina T, Myshakina NS, Parniak MA, Wang Z. Clicking 3’-Azidothymidine into Novel Potent Inhibitors of Human Immunodeficiency Virus. J Med Chem. 2013;1(56):8765–80.

22. Kolb HC, Finn MG, Sharpless KB. Click Chemistry: Diverse Chemical Function from a Few Good Reactions. Angew Chemie - Int Ed. 2001;40(11):2004–21.

23. Jiang X, Hao X, Jing L, Wu G, Kang D, Liu X, et al. Recent applications of click chemistry in drug discovery. Expert Opin Drug Discov [Internet]. 2019;14(8):779–89. Available from: https://doi.org/10.1080/17460441.2019.1614910

24. Alencar D, Gonçalves J, Cerqueira SA, Soares H, Petronilho A. Development of Triazoles based on AZT and their Anti-Viral Activity Against HIV-1. bioRxiv. 2019;

CAPÍTULO 4: Principais resultados: implicações clínicas e para a saúde pública

Nesta tese, apresentamos os resultados de estudos epidemiológicos realizados com mulheres grávidas recém-diagnosticadas com o VIH em Luanda, a cidade capital de Angola. Além disso, apresentamos resultados de um estudo experimental realizado *in vitro* para avaliação da eficácia de novos compostos na inibição da replicação viral do VIH-1. A apresentação, análises e discussão dos resultados obtidos nestes estudos, foram agrupados em três tópicos principais e apresentados como se segue:

1. **Prevalência do VIH, coinfecções e fatores de risco de infeção em mulheres grávidas de Luanda**

Os resultados obtidos neste estudo mostraram que 2,6% de um total de 1612 mulheres grávidas inscritas nos cuidados pré-natal da Maternidade Lucrécia Paim, entre abril e junho de 2018, estavam infetadas com o VIH. A prevalência do VIH observada em mulheres grávidas do nosso estudo foi menor comparado com a prevalência observada em outros estudos realizados com mulheres grávidas residentes na Etiópia (5,5%)[118], Tanzânia (5,6%)[119] e Camarões (6%)[120], mas a prevalência do nosso estudo foi alta quando comparado com as mulheres grávidas residentes na Índia (0,88%)[121] e Brasil (0,09%)[122]. Tendo em conta as caraterísticas socioeconómicas da população residente em Angola, o crescente movimento interno e deslocação para países com elevada prevalência do VIH, estima-se que haja um alastramento da epidemia do VIH/SIDA e outros agentes causadores de doenças infeciosas a nível nacional nos próximos anos. Quanto as coinfecções, 13% das mulheres grávidas seropositivas para o VIH testaram positivo para mais de um marcador de doenças infeciosas. O vírus da hepatite B e a sífilis estiveram presentes em 7,5% e 5,0%, respetivamente. Por outro lado, nenhuma gestante testou positiva na serologia para o vírus da hepatite C. Além disso, 94,4% e 11,1% das gestantes positivas para o VIH, apresentaram IgG e IgM contra o vírus da dengue, respetivamente. A infeção recente de dengue (IgG-/IgM+ ou IgG+/IgM+) esteve presente em 11,1% das gestantes seropositivas para o VIH, enquanto a infeção passada (IgG+/IgM-) esteve presente em 83,3%. A infeção recente pelo vírus da dengue observado em mulheres grávidas deste estudo mostra que Luanda pode ser uma região endémica para o vírus da dengue com elevado risco de disseminação de doenças transmitidas pela picada de mosquitos[123,124].

A prevalência de hepatite B (7,5%) observada em mulheres grávidas infetadas com o VIH neste estudo é consistente com a prevalência de coinfecção observada em mulheres da região da África subsariana (6–25%)[125]. Os nossos resultados foram semelhantes à prevalência observada em

mulheres grávidas dos Camarões (7,7%)[126], alta em relação à prevalência observada em mulheres grávidas da Nigéria (0,5%)[127], Botsuana (3,1%)[128], Ruanda (4,1%)[129] e Sudão (5,6%)[130], mas inferior quando comparado com a prevalência observada em mulheres grávidas da Etiópia (12,1%)[118] e Gana (14,9%)[131]. A prevalência de coinfecção do VIH com o vírus da hepatite C tem sido baixa (3,3%) no sul de África comparado com a prevalência observada na região norte de África (42,3%)[132]. Por outro lado, a prevalência de coinfecção do VIH com a sífilis foi maior comparada aos resultados observados em mulheres grávidas do Uganda (0,52%)[133] e Tanzânia (0,9%)[134].

Os resultados da serologia deste estudo indicam que além do VIH, mais atenção deve ser dada para o vírus da hepatite B, sífilis e dengue, que também são frequentes em mulheres residentes em Angola e que poderiam impactar negativamente o sucesso da gravidez. Por outro lado, embora nenhum caso de hepatite C tenha sido identificado nas gestantes deste estudo, os programas de controlo de doenças infeciosas de Angola devem continuar em alerta e reforçar o rastreio das hepatites virais, sífilis e doenças transmitidas por vetores, sobretudo em mulheres grávidas residentes em Luanda. Estas recomendações são fortemente suportadas considerando que recentemente a Organização Mundial da Saúde (OMS) recomendou o rastreio integrado do VIH, hepatite B e sífilis pelo menos uma vez durante os cuidados pré-natais sobretudo nas regiões endémicas para garantir o diagnóstico e tratamento precoce, e desse modo, evitar a transmissão vertical e alastramento da infeção a nível local[135].

O rastreio do VIH e coinfecção com outras doenças sexualmente transmissíveis (DST) em mulheres grávidas é um bom indicador da distribuição de casos de infeção na população. Além disso, estes estudos servem para estimar a taxa de transmissão vertical[136]. Em Angola, o início da atividade sexual é precoce tanto em homens como em mulheres. Os estudos realizados em Angola mostraram que 68% e 69% das mulheres e homens dos 20–49 anos, respetivamente, iniciam a atividade sexual antes dos 15 anos e até aos 20 anos, já têm pelo menos um ou mais filhos[101]. Contrariamente ao descrito nos estudos anteriores, o nosso estudo mostrou gestantes com menos de 15 anos, indicando que o início da atividade sexual em Luanda, pode ser ainda mais precoce. A idade das gestantes inscritas no nosso estudo estava entre 12–45 anos com média de idade de 27±7. A representatividade das gestantes infetadas com o VIH/SIDA do nosso estudo, foi conseguida mediante a parceria obtida com o programa de prevenção de transmissão vertical do VIH, que garantiu a assistência das gestantes incluídas neste estudo durante o período pré-natal na Maternidade Lucrécia Paim. O recrutamento destas gestantes nesta unidade sanitária de referência

nacional para o cuidado da saúde materna e dos recém-nascidos, permitiu a inclusão em torno de 70–80% do total de gestantes infetadas com o VIH/SIDA em Luanda, durante o período do estudo.

As gestantes diagnosticadas com o VIH neste estudo, desconheciam o seu estado serológico e nenhuma se encontrava submetida ao TARV. Assim sendo, e com base nas recentes recomendações da OMS de iniciar o tratamento logo após o diagnóstico, as gestantes positivas para o VIH iniciaram o TARV com o esquema preferencial usado em Angola, que inclui o TDF (300 mg), 3TC (300 mg) e EFV (600 mg)[28]. Além disso, as gestantes deste estudo foram seguidas até o momento do parto e receberam AZT (300 mg) para impedir a transmissão vertical do VIH, conforme o protocolo de prevenção da transmissão vertical do VIH estabelecido em Angola. Após o nascimento, os recém-nascidos expostos ao VIH receberam NVP em xarope durante as primeiras 6 semanas[28].

Devido à perda de seguimento das gestantes infetadas com o VIH, os estudos de acompanhamento de crianças expostas aos VIH-1 não têm sido bem-sucedidos em Angola. Por esta razão, futuros estudos sobre acompanhamento de crianças expostas ao VIH-1 em diferentes regiões de Angola, devem ser realizados para avaliação do tempo de sororreversão e os fatores maternos ou neonatais determinantes para a sororreversão.

Apesar de existir o protocolo de rastreio do VIH nas unidades sanitárias de seguimentos a grávidas, algumas gestantes ainda têm a elucidação diagnóstica da infeção pelo VIH apenas no final da gestação, o que muitas vezes impede a aplicação completa do protocolo de prevenção da transmissão vertical do VIH. Assim sendo, é necessário a realização de campanhas de sensibilização contínuas para incentivar as mulheres jovens a realizarem o rastreio de doenças infeciosas antes e durante a gravidez, de modo a poderem saber da sua condição serológica e beneficiarem de um tratamento adequado e garantir o sucesso da gravidez. Além disso, deve-se criar condições para se oferecer o teste de resistência a todas as mulheres grávidas infetadas pelo VIH, para ajudar a reduzir as hipóteses de transmissão vertical do vírus. O custo-benefício da realização do teste de resistência antes do início do TARV seria importante, uma vez que, com a realização dos testes de resistência, o paciente irá iniciar o TARV com o esquema adequado, reduzindo as hipóteses de progressão da doença para SIDA, falha terapêutica ou transmissão vertical, no caso das mulheres grávidas.

O nosso estudo também avaliou os determinantes sociodemográficos de infeção do VIH em gestantes de Luanda. Os resultados mostraram que as gestantes com idades abaixo dos 25 anos estão protegidas para contraírem a infeção pelo VIH (AOR: 0,43; p=0,026), apesar de o mesmo grupo

mostrar maior vulnerabilidade para contrair o vírus da hepatite B ou sífilis (AOR: 1,33; p=0,868). Além disso, o grupo de gestantes abaixo dos 25 anos também apresentaram vulnerabilidade elevada para contrair o vírus da dengue (OR: 13,0; p=0,039).

A coexistência de doenças infeciosas em mulheres jovens residentes na província de Luanda não é surpreendente, visto que grande proporção da população de Luanda apresenta vulnerabilidade socioeconómica, vivem em bairros de lata ou moradias com limitado acesso aos cuidados de saúde e saneamento básico. Outro fator importante de aumento do risco para contrair doenças infeciosas em Luanda, é o crescimento populacional e o agravamento das condições socioeconómicas. Segundo os resultados definitivos do senso populacional realizado em Angola no ano 2014, a população residente na província de Luanda aumentou e até 2014 contava com cerca de 7 milhões de habitantes[137]. Este crescimento populacional associado ao início precoce da atividade sexual, elevada taxa de desemprego na população jovem, baixo nível de escolaridade, pobreza extrema associado a distribuição inadequada dos recursos, podem ser fatores que têm contribuído para o aumento de potenciais grupos de disseminação de doenças infeciosas em Luanda e indicam que as doenças infeciosas podem se alastrar ainda mais por todo o país nos próximos anos[101]. Por outro lado, o comércio internacional, viajem de pessoas de e para países endémicos ou com transmissão ativa de agentes infeciosas, urbanização descontrolada, saneamento básico debilitado e debilidade observado nas infraestruturas de saúde, podem também ajudar a explicar o aumento de casos de DST, dengue e outras arboviroses na cidade capital de Angola[138–140]. Entretanto, a implementação de programas de rastreio serológico ou molecular a todos os viajantes e comerciantes internacionais sobretudo aqueles que viajam para Luanda devido ao turismo ou comercialização do petróleo, seria crucial para ajudar a controlar e reduzir o número de casos importados de agentes infeciosos em Angola. Esta recomendação é suportada pelo facto de estudos moleculares mostrarem que as linhagens do vírus da dengue circulante em Angola desde o final de 1968, pertencem às linhagens circulantes no continente Americano e em algumas partes do continente Africano, indicando que as linhagens do vírus da dengue que circula em Angola foram possivelmente importadas destas regiões[124].

2. Epidemiologia molecular do VIH-1 em Angola

Os estudos de epidemiologia molecular do VIH-1 em Angola têm revelado elevada diversidade genética do VIH-1 com presença de mutações de resistência contra os ITRN e os ITRNN[105–113]. O

nosso estudo gerou as primeiras sequências do fragmento do gene *pol* depositadas do GenBank (número de acesso MK543512 para MK543545) de pacientes infetados com o VIH-1 em Angola, sequenciados a partir de uma metodologia de genotipagem da região da protéase e transcriptase reversa, implementado no Instituto Nacional de Investigação em Saúde (INIS), localizado em Luanda. Esta é uma técnica confiável usada com sucesso neste estudo, representando um avanço importante para o estudo da diversidade genética do VIH-1 e para o mapeamento de mutações associadas à resistência aos ARVs usados em Angola. Acreditamos que a implementação desta metodologia como um teste de rotina em todos os pacientes infetados com o VIH-1, sobretudo as mulheres grávidas, pode ser uma medida importante capaz de permitir a identificação precoce dos subtipos resistentes aos ARVs e permitir a introdução de medidas profiláticas capazes de controlar efetivamente a infeção em gestantes e reduzir a transmissão de subtipos do VIH-1 com resistência aos ARVs utilizados em Angola.

Os nossos resultados indicaram possível alteração do perfil epidemiológico dos subtipos do VIH-1 em Angola nos últimos anos. Num contexto de predomínio dos subtipos C e F1, identificámos o aumentou significativo ($p<0,001$) de subtipos recombinantes de 23,6% em 2000 para 41,4% em 2019. Com o aumento da população migrante desde 2002, data do fim da guerra civil em Angola, muitos indivíduos regressaram para Angola, o que pode ser um dos fatores que contribuiu para introdução de novos subtipos do VIH-1 e o aumento da diversidade genética do vírus em Angola. As variantes do subtipo C circulante em Angola foram semelhantes aos isolados em pacientes do Botsuana, Moçambique, Tanzânia e África do Sul, indicando múltipla introdução do subtipo C em Angola. Estudos documentam que o subtipo C parece ser menos patogénico, causando uma infeção com menor cronicidade, o que leva o indivíduo infetado a permanecer assintomático por muito tempo, favorecendo a transmissão e disseminação da doença localmente[38]. Por outro lado, o subtipo F1 formou um agrupamento monofilético com subtipos isolados de amostras de pacientes do Brasil, o que pode indicar uma única introdução deste subtipo e o efeito fundador desta variante do VIH-1 da América do Sul em Angola.

O predomínio do subtipo C no sul de Angola e o subtipo F1 no norte de Angola, tem proporcionado eventos de recombinação e o surgimento de mosaicos F1/C na região central, o que reflete maior fluxo de pessoas do norte e sul para a região central de Angola, sobretudo em Luanda, a capital do país. Além disso, o predomínio atual do subtipo C na região central de Angola, pode ser uma indicação da existência de uma rede de transmissão favorável do VIH-1 na capital do país em

detrimento do aumento do fluxo de indivíduos provenientes da região sul de Angola. Entretanto, os resultados deste estudo enfatizam a necessidade da realização de investigação da diversidade genética do VIH-1 em grupos específicos como trabalhador sexual, HSH, camionistas e prisioneiros, sobretudo os provenientes da região sul ou norte de Angola, para entendermos a origem e as principais rotas de transmissão dos subtipos C e F1, em Angola. Além disso, é necessário investigar se estamos perante a presença de novas estirpes C e F1 com maior ou menor infecciosidade, ou se o vírus encontrou condições mais favoráveis que potenciam a disseminação mais rápida em Luanda. Atualmente, não existe uma origem exata do subtipo C circulante em Angola, apesar de alguns estudos indicarem introdução múltipla de variantes do subtipo C conforme mostrado também no nosso estudo, em resultado do aumento da população imigrante proveniente dos países da região sul de África, desde o final dos anos 1970 até os anos 2000[141].

Diferente do subtipo C que manteve a sua frequência em torno de 20% de 2000 para 2019, o subtipo F1 aumentou de 9,4% em 2000 para 14,1% em 2019. Os fatores que ocasionaram este aumento na frequência do subtipo F1, são desconhecidos e carecem de investigação nos futuros estudos. O subtipo F1 provavelmente foi introduzido na região norte de Angola em 1959[114,115], e desde então, é um dos subtipos predominantes na epidemia do VIH-1 em Angola[110]. Entre 2000–2019, houve aumento do subtipo A e redução dos subtipos A1, A2, G, H, J e K. Esta alteração no perfil epidemiológico do VIH-1 em Angola pode representar importantes consequências sobretudo para o diagnóstico, monitoramento, tratamento e gestão da pandemia do VIH/SIDA em Angola. A CRF02_AG foi a variante recombinante mais frequente encontrada a circular em Angola, enquanto a CRF01_AE foi observada a baixa prevalência. A elevada prevalência da CRF02_AG é uma forte indicação do fluxo de pessoas residentes em Angola com os países da África subsariana que têm apresentado maior frequência da CRF02_AG. Por outro lado, a origem da variante CRF01_AE circulante em Angola precisa de mais investigação de natureza filogenética para esclarecer a sua origem, apesar de alguns estudos indicarem que a CRF01_AE foi introduzida em Angola entre 2002–2006, resultante do aumento do fluxo da população imigrante de origem asiática para Angola, sobretudo a população chinesa que conta com mais de 20 000 indivíduos residentes em Angola, e que apresenta a variante CRF01_AE como sendo um dos subtipos mais prevalentes daquele país asiático.

Os ITR fazem parte do esquema preferencial do TARV em Angola e em muitos outros países em desenvolvimento[28]. Entretanto, os nossos resultados mostraram que 18% das mulheres grávidas

naïve tinham mutações relacionadas com a redução da eficácia dos ITRN e ITRNN. A semelhança dos estudos anteriores[110–113], identificamos as mutações K103N, G190A, Y181I e P225H contra a classe do ITRNN, e as mutações M41L, D67N, T69D e T215S contra os ITRN. Em África, as mutações K103N, G190A e Y181C são as mais frequentes na classe dos ITRNN, enquanto a mutação M184V é a mais frequente na classe dos ITRN[91]. A mutação M184V reduz a suscetibilidade viral ao fármaco 3TC e aumenta a suscetibilidade viral aos fármacos TDF e AZT, as mutações K103N e G190A reduzem a suscetibilidade viral aos fármacos EFV e NVP, e finalmente a mutação Y181C reduz a suscetibilidade viral aos fármacos de segunda geração, a ETV e RPV[92,93]. Entre os anos 2000–2019, Angola registou aumento na frequência das mutações M184V (de 50% para 64,3%), K103N (de 34,5% para 42,3%), G190A (de 17,2% para 46,2%), e redução na frequência da mutação Y181C (de 17,2% para 7,7%). O aumento da circulação destas mutações pode ser o resultado do uso irregular e não monitorizados dos esquemas de TARV contendo os ITR em Angola[28]. Portanto, o aumento da frequência da mutação M184V em Angola, indica que esquemas de TARV contendo TDF ou AZT poderiam ser os mais apropriados para o combate da pandemia do VIH/SIDA em Angola. Além disso, a presença da mutação M184V pode ser um fator importante que favorece o sucesso do protocolo de prevenção da transmissão vertical do VIH, devido ao aumento da suscetibilidade viral ao fármaco AZT que é usado durante o parto em todas as mulheres grávidas seropositivas para o VIH, para evitar a transmissão vertical. O perfil de mutações observados neste estudo, com maior prevalência observado na mutação M184V indica que um número significativo de pacientes infetados pelo VIH-1 em Angola, encontram-se em monoterapia com TDF ou AZT e que podem experimentar falha terapêutica no futuro. Além disso, em Angola existe a circulação de vírus com mutações de resistência aos análogos à timidina (D67N, K70R, K219E, L210W, M41L e T215Y/F) que podem causar resistência cruzada entre os ARVs da classe dos ITRN, o que representa uma ameaça significativa para o sucesso do TARV no país[92].

Nos últimos anos, um grande número de investigadores documentaram que os determinantes sociodemográficos, comportamentais e clínicos podem desempenhar um papel crucial na disseminação de variantes do VIH-1 com mutações de resistência aos ARV principalmente nos países de baixa e média renda[142,143]. Entretanto, os nossos resultados embora não tenham significância estatística, indicaram maior vulnerabilidade para o surgimento de mutações de resistência em gestantes das regiões não urbanizadas (AOR: 2,73; p=0,247), em gestantes com elevado nível de

escolaridade (AOR: 6,27; p=0,086), em gestantes com comorbilidade (AOR: 5,47; p=0,261) e em gestantes infetadas com algum subtipo do VIH-1 diferente do subtipo C (AOR: 1,60; p=0,619).

A identificação das características sociodemográficas que afetam a disseminação de variantes resistentes do VIH-1 pode ser um alvo de intervenção importante para controlar a pandemia do VIH/SIDA e garantir o sucesso do TARV em Angola. O rastreio de comorbilidades em mulheres grávidas infetadas com o VIH-1 deveria ser priorizado para garantir melhores resultados no uso do TARV e evitar a transmissão de doenças infeciosas para os recém-nascidos. Além disso, é necessário reforçar a educação sanitária sobre o VIH/SIDA para garantir maior adesão ao TARV e reduzir atitudes discriminatórias em relação às pessoas que vivem com o VIH[20].

A implementação do teste de resistência aos ARVs como rotina em todos os pacientes infetados com o VIH, seria uma estratégia bastante útil para reforçar a monitorização da epidemiologia molecular do VIH-1, a taxa de resistência aos ARVs e para otimização do TARV em Angola. A identificação de subtipos do VIH-1 com mutações de resistência em mulheres grávidas recém-diagnosticadas em Luanda, é uma forte indicação de que a transmissão de vírus resistentes é frequente em Angola, e que o mesmo cenário pode estar a acontecer nas outras regiões do país. Entretanto, é necessário a realização de mais estudos noutras regiões de Angola, para a identificação da taxa de transmissão de vírus resistentes fora de Luanda. O aumento do acesso aos ARVs, o uso irregular e não monitorizado dos ARVs, a falta de adesão ao TARV muitas vezes associados as dificuldades socioeconómicas dos pacientes, o aumento de imigrantes potencialmente infetados com VIH, a deslocação de pacientes angolanos para países que têm o TARV implementado a longo tempo, são fatores que ajudam a explicar a origem dos subtipos com mutações de resistência em Angola[143,144]. Além disso, a circulação de vírus resistentes aos ARVs da classe dos ITRN e ITRNN em pacientes não expostos aos ARVs é uma forte indicação da necessidade do Ministério da Saúde de Angola considerar a possibilidade de atualizar os regimes de TARV e incluir os inibidores de protéase ou integrase nos esquemas de primeira linha do tratamento do VIH/SIDA em Angola[70].

O nosso estudo mostrou que nos últimos 20 anos (2000−2019), apenas um paciente apresentou a mutação na posição I54M que confere resistência aos inibidores de protéase, o que sugere que esta classe de ARV pode ser usados com sucesso para o tratamento do VIH-1 em pacientes com falha virológica ou imunológica[28,103]. Estas medidas com a realização da genotipagem do VIH-1 em todos

os pacientes antes da exposição aos ARVs poderá ter um impacto socioeconómico positivo em Angola, sobretudo na redução da transmissão vertical de estirpes do VIH-1 resistentes aos ARVs, a personalização da TARV e redução dos custos relacionados ao tratamento de pacientes infetados com o VIH/SIDA no país.

3. Descoberta de novas drogas contra o VIH-1

Embora o acesso universal a TARV tenha contribuído para a redução da morbilidade e mortalidade associada ao VIH/SIDA, em todo o mundo, a disseminação de variantes resistentes até mesmo contra os ARVs de nova geração nos países em desenvolvimento, constitui uma ameaça para a erradicação do VIH/SIDA até 2030[74,145]. Assim sendo, a descoberta de novos compostos com eficiência aumentada, efeitos colaterais reduzidos e com pouca ou nenhuma citotoxicidade, tem se tornado um aspeto importante para o combate da pandemia do VIH/SIDA. Atualmente, existem numerosos compostos sintetizados com base na modificação química do grupo 3'-Azido do AZT, que se encontram em diferentes etapas de ensaios experimentais, e alguns compostos têm mostrado resultados promissores na inibição da replicação do VIH-1[77–81]. Os estudos demostram que a introdução de grupos aromáticos volumosos e longa cadeia de hidrocarboneto na estrutura química do AZT pode resultar em compostos com atividade antiviral aumentada e que poderiam ser usados como uma alternativa ao AZT no tratamento ou profilaxia da infeção[82]. Neste estudo, rastreamos a eficácia antiviral contra o VIH-1 e toxicidade de onze novos compostos com modificação química do grupo 3'-Azido do AZT. Os compostos avaliados neste estudo foram sintetizados com a técnica de "química de cliques" que permitiu a introdução de cadeias aromáticas, hidrocarbonetos e sais, como descrito e apresentado por Alencar e colaboradores[146]. A estrutura dos compostos, bem como, os resultados obtidos estão detalhados no manuscrito 6 (**Antiretroviral activity of novel 3'-Azidothymidine triazole derivatives against wild-type HIV-1 *in vitro***) deste documento.

Após a reação química para a síntese desses derivados do AZT, os compostos foram dissolvidos em 10mM, armazenados a -20°C e posteriormente testados em células mononucleares do sangue periférico que foram isolados a partir do sangue de dadores saudáveis. Antes dos ensaios *in vitro* de avaliação da atividade antiviral e citotoxicidade, um milhão de células eram cultivadas por 7 dias a 37°C, usando o meio de cultura RPMI na presença de interleucina 2 (IL-2) em uma atmosfera suplementada com 5% de CO_2. Além disso, o meio de cultura era suplementado com 10% de soro fetal bovino, 1% de penicilina/estreptomicina e 1% de L-glutamina. A atividade antiviral contra o

VIH-1, bem como a citotoxidade dos compostos, foram avaliadas em dois diferentes métodos de tratamentos, utilizando células não pretratadas e células pretratadas com os compostos. As células pretratadas foram colhidas 12 horas após a cultura, centrifugadas e ressuspendidas em outro meio de cultura RPMI antes da infeção. Todas as células foram infetadas com a estirpe HIV-1 NL 4-3 marcado com proteína verde florescente na presença de polybreno (8 μg/ml). Durante a infeção, as células foram centrifugadas a 2500 rpm durante 90 minutos a temperatura ambiente e mantidas em cultura por 2 horas. Posteriormente, as células eram lavadas para remover as partículas virais não ligadas, ressuspendidas em 1 mL de meio RPMI na presença dos compostos e cultivados durante 5 dias. Todos as experiências foram conduzidas na presença de um grupo de células não infetadas que serviu como controlo negativo de infeção, um grupo de células infetadas não tratadas que serviu como controlo positivo de infeção e um grupo de células infetadas e tratadas com o AZT que serviu como controlo da eficácia dos compostos. Inicialmente, todos os compostos foram rastreados com uma concentração de 10μM e resposta a dose (20–100μM) foi avaliado apenas nos compostos com resultados promissores. A avaliação da infeção e ação antiviral dos compostos foi feito através de citometria de fluxo. Adicionalmente, todas as células foram marcadas com 7-AAD antes das análises para a avaliação da viabilidade celular e exclusão das células mortas.

Resultados de cinco experiências independentes mostraram que as células tinham viabilidade celular acima dos 90% após a infeção em ambos os métodos de tratamento, pretratados e não pretratados. Para controlar e garantir a viabilidade dos compostos testados, foi realizado uma experiência na ausência de infeção para excluir a possibilidade de existência de algum composto tóxico para as células na concentração de 10μM. Entretanto, comparado com o AZT, nenhuma toxicidade foi observada quando os compostos foram adicionados na ausência da infeção, indicando que os compostos eram viáveis e que não interferiam com o normal funcionamento celular. Por outro lado, cinco experiências independentes foram realizadas para avaliação da capacidade inibitória do ciclo de replicação do VIH-1. Os resultados obtidos mostraram que quase todos (91%, 10/11) os compostos foram capazes de reduzir a infeção no método de tratamento com células não pretratadas, enquanto todos os compostos reduziram a infeção no método de tratamento com células pretratadas, quando foram comparados com o controlo positivo de infeção ou células que foram infetadas e não tratadas. Embora os compostos apresentaram viabilidade celular acima dos 80% nos dois métodos de tratamento, os nossos resultados mostraram que estes compostos são

mais eficazes num cenário de pré-tratamento, podendo desempenhar importante papel no controlo da infeção no contexto de profilaxia de pré exposição.

Dos onze compostos rastreados para a atividade antiviral contra o VIH-1 e citotoxicidade, os compostos 2 (1,4-triazol) e 6 (1,5-triazol) foram os que apresentaram resultados promissores na inibição da replicação viral com baixa ou nenhuma toxicidade na concentração de 10µM, sobretudo nas células pretratadas. Os restantes derivados apresentaram baixa capacidade de inibição viral em ambos os métodos de tratamento, utilizando células não pretratadas ou utilizando células pretratadas, apesar de manterem elevada viabilidade celular em ambos os métodos de tratamento. Assim sendo, quatro experiências independentes para a avaliação de resposta a dose (20–100µM) foram conduzidas com estes 2 compostos promissores usando o método de tratamento de células pretratadas por mostrar ser o método mais eficiente na inibição viral utilizando estes compostos. A viabilidade dos compostos utilizando elevadas dosagens (20–100µM) não foi avaliado e deve ser considerado nos futuros estudos. Os resultados dos experimentos mostraram que os dois compostos promissores tinham a capacidade para reduzir mais de 50% da infeção em todas as dosagens (20–100µM) testadas, apesar de a citotoxicidade do composto 6 (1,5-triazol) aumentar com o aumento da concentração do composto. Estes resultados sugerem que a atividade inibitória deste composto na presença de elevadas dosagens (20–100µM) pode resultar na morte direita das células infetadas com o VIH-1. Entretanto, futuros estudos devem avaliar se a morte celular é decorrente da elevada concentração e toxicidade do composto. Contrariamente ao observado no composto 6 (1,5-triazol), o composto 2 (1,4-triazol) apresentou resultados satisfatórios na inibição viral, além de manter a viabilidade celular mesmo na presença de elevadas dosagens (20–100µM). A dose efetiva do composto 2 (1,4-triazol) foi observada na concentração de 40µM, enquanto a dose efetiva do composto 6 (1,5-triazol) foi observada na concentração de 80µM. Estes resultados indicam que o composto 2 (1,4-triazol) tem grande afinidade com as enzimas celulares e com a enzima transcriptase reversa do VIH-1, devido a sua eficiência na inibição viral e viabilidade celular em pequena ou elevada dosagem. Por outro lado, o composto 6 (1,5-triazol) precisou de elevadas concentrações do composto para garantir a inibição viral o que causou a morte das células infetadas. Os resultados deste composto difere com as abordagens atuais de busca por compostos antivirais ativos que recomendam que devem ter atividade antiviral a baixa dosagem e com pouca ou nenhuma toxicidade[77–82]. A elevada concentração deste composto parece reduzir a afinidade com as enzimas celulares o que por sua vez, impede a formação de análogos trifosfatos ativos capazes

de inibir a atividade da enzima transcriptase reversa do VIH-1. Entretanto, futuros estudos de avaliação da interação desde composto com as enzimas celulares devem ser consideradas de modo a permitir a otimização da concentração eficaz do composto para atividade antiviral contra o VIH-1. A dose efetiva do AZT usado como controlo da eficácia dos compostos também foi observado na concentração de 40µM, o que é similar ao observado no composto 2 (1,4-triazol) e que poderia indicar que este composto derivado da modificação química do grupo 3'-Azido do AZT pode ser usado como uma alternativa ao AZT para o uso profilático num cenário de pré-exposição ou pós-exposição.

Conforme mostrado por Alencar e colaboradores[146], os compostos com resultados promissores deste estudo, apresentam cadeias aromáticas volumosas com diferentes padrões de substituição nas posições 1,4- e 1,5-triazol. Por outro lado, os compostos contendo grande volume da cadeia aromática, cadeia curta ou longa de hidrocarbonetos, não apresentaram resultados satisfatórios quanto a atividade antiviral contra o VIH-1. Além disso, era esperado que um grande volume aromático ou cadeia de hidrocarbonato muito longa na posição 1,4-triazol, 1,5-triazol ou em ambas as posições, teria eficácia antiviral contra o VIH-1, entretanto, os nossos resultados mostraram que a presença de grandes volumes de cadeias aromáticas ou cadeias de hidrocarbonetos bastantes longas nestas posições, podem reduzir a atividade antiviral do composto, aumentar a toxicidade e causar a morte direita das células infetadas com o VIH-1. Estes resultados mostraram que a modificação do grupo 3'-Azido do AZT é bastante complexa e que mais estudos devem ser realizados para a identificação e definição dos padrões de substituição na estrutura química do AZT.

A baixa atividade antiviral em derivados triazoles do AZT também foram reportados em outros estudos de síntese e descoberta de compostos antivirais contra o VIH-1[82]. Um dos fatores envolvidos na perda da atividade antiviral e aumento da citotoxicidade dos compostos deste estudo pode ser o facto de os compostos com atividade inibitória da enzima transcriptase reversa do VIH-1, precisarem de várias etapas de fosforilação pelas enzimas kinase e fosfotransferases da célula do hospedeiro antes da formação de análogos trifosfatos ativos[62,147]. Portanto, a falta de atividade antiviral e o aumento no nível de citotoxicidade observado nestes compostos, pode ser atribuída à ineficiente fosforilação intracelular ou pouca afinidade dos compostos na presença de baixa concentração (10µM) com a enzima transcriptase reversa do VIH-1[62–64,147]. Assim sendo, estudos aprofundados sobre a metabolização celular dos novos compostos derivados da modificação química do grupo 3'-Azido do AZT, bem como a avaliação do seu efeito na atividade antiviral contra

o VIH-1 e citotoxicidade são necessários para melhorar o processo de síntese de novos compostos capazes de combater agentes infeciosos virais. Além disso, os compostos com propriedades farmacológicas atraentes devem ser testados em estirpes com mutações de resistência ao AZT ou estirpes com mutações de resistente contra outras classes de ARVs, para a avaliação da sua utilidade clínica no tratamento de pacientes com falha terapêutica ou que apresentam mutações de resistência contra os ARVs[63,147].

CAPÍTULO 5: Conclusões e direção para futuros estudos

Os resultados desta tese de doutoramento mostram que o rastreio de doenças infeciosas durante os primeiros meses do pré-natal, é uma janela de oportunidade importante para diagnosticar precocemente o VIH e outros agentes infeciosos em mulheres grávidas de Angola e permitir a implementação atempada de medidas para prevenir a transmissão vertical. O VIH, hepatite B, sífilis e dengue são frequentes em mulheres grávidas de Luanda, a cidade capital de Angola. As mulheres com idades abaixo dos 25 anos foram as que apresentaram maior risco para contrair estas doenças infeciosas. Dificuldades socioeconómicas, comportamentos sexuais de risco e início precoce da atividade sexual, são fatores que potenciam a infeção e disseminação de doenças infeciosas em mulheres jovens de Luanda.

A epidemia do VIH-1 em Angola é dominada pelos subtipos C, F1, CRF02_AG e recombinantes U/H. Futuros estudos de sequenciação completa do genoma do recombinante U/H são necessários para elucidar se estamos perante um novo CRF_U/H não reconhecido anteriormente. As mutações M184V, G190A, K103N e Y181C na região da TR foram as mais frequentes detetadas nestas mulheres grávidas não expostas aos ARVs. Baixa frequência de mutações de resistência na região da protéase foi observada nos últimos 20 anos em Angola, mostrando que os inibidores da protéase ou integrase deveriam ser incluídos nos esquemas de primeira linha de TARV para reduzir o cenário da circulação de estirpes do VIH-1 resistentes aos ARVs em Angola.

Identificamos um novo composto com modificação química do grupo 3'-Azido que apresentou resultados promissores contra o VIH-1 com baixa ou nenhuma citotoxicidade nas concentrações entre 10–100µM. As avaliações deste composto promissor em estirpes resistentes ao AZT ou outros ARVs, não foram feitos e devem ser considerados nos próximos estudos. Além disso, mostramos que o aumento do volume da cadeia aromática ou longa cadeia de hidrocarbonetos pode levar a perda da atividade antiviral do composto, aumentar a toxicidade e matar as células infetadas com o VIH-1.

REFERÊNCIAS BIBLIOGRÁFICAS

1. Etienne L, Delaporte E, Peeters M. Origin and Emergence of HIV/AIDS [Internet]. First Edit. Elsevier Inc.; 2011. Available from: http://dx.doi.org/10.1016/B978-0-12-384890-1.00026-1
2. CDC. Epidemiologic Aspects of the Current Outbreak of Kaposi's Sarcoma and Opportunistic Infections. N Engl J Med 1982;
3. Greene WC. A history of AIDS: Looking back to see ahead. Eur J Immunol 2007;37(SUPPL. 1):94–102.
4. De Cock KM, Jaffe HW, Curran JW. Reflections on 30 Years of AIDS. Emerg Infect Dis 2011;17(6):1044–8.
5. Hogan CA, Iles J, Frost EH, et al. Epidemic History and Iatrogenic Transmission of Blood-borne Viruses in Mid-20th Century Kinshasa. J Infect Dis 2016;214(3):353–60.
6. MMWR. First Report of AIDS. CDC [Internet] 2001;50(21). Available from: https://www.cdc.gov/mmwr/pdf/wk/mm5021.pdf
7. Barré-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science (80-) 1983;220:868–71.
8. Vahlne A. A historical reflection on the discovery of human retroviruses. Retrovirology 2009;6:1–9.
9. Henrickson R V., Maul DoH, Osborn KG, et al. Epidemic of Acquired Immunodeficiency in Rhesus Monkeys. Lancet 1983;388–90.
10. Daniel MD, Letvin NL, King NW, et al. Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. Science (80-) 1985;228(4704):1201–4.
11. Sharp PM, Shaw GM, Hahn BH. Simian Immunodeficiency Virus Infection of Chimpanzees. J Virol 2005;79(7):3891–902.
12. Sharp PM, Hahn BH. Origins of HIV and the AIDS pandemic. Cold Spring Harb Perspect Med 2011;1(1):a006841.
13. Silvestri G, Paiardini M, Pandrea I, Lederman MM, Sodora DL. Science in medicine Understanding the benign nature of SIV infection in natural hosts. J Clin Invest 2007;117(11):3148–54.
14. Hahn BH, Shaw GM, Cock KM De, Sharp PM. AlDS as a Zoonosis : Scientific and Public Health Implications. Sci compass Rev 2000;287(January).
15. Paraskevis D, Nikolopoulos GK, Magiorkinis G, Hodges-Mameletzis I, Hatzakis A. The application of HIV molecular epidemiology to public health. Infect Genet Evol [Internet]

2016;46:159–68. Available from: http://dx.doi.org/10.1016/j.meegid.2016.06.021

16. Vidal N, Peeters M, Mulanga-Kabeya C, et al. Unprecedented Degree of Human Immunodeficiency Virus Type 1 (HIV-1) Group M Genetic Diversity in the Democratic Republic of Congo Suggests that the HIV-1 Pandemic Originated in Central Africa. J Virol 2000;74(22):10498–507.
17. Pépin J. The origins of AIDS: From patient zero to ground zero. J Epidemiol Community Health 2013;67(6):473–5.
18. Sauter D, Kirchhoff F. Key Viral Adaptations Preceding the AIDS Pandemic. Cell Host Microbe [Internet] 2019;25(1):27–38. Available from: https://doi.org/10.1016/j.chom.2018.12.002
19. UNAIDS. 2020 Global AIDS report. 2020 Glob AIDS Rep 2020;1:380.
20. Unaids. Unaids Data 2019. Unaids [Internet] 2019;Available from: https://www.unaids.org/en/resources/documents/2019/2019-UNAIDS-data
21. UN General Assembly. Political Declaration on HIV and AIDS: On the Fast-Track to Accelerate the Fight against HIV and to End the AIDS Epidemic by 2030. New York [Internet] 2016;17020(June):1–27. Available from: http://web.ua.es/es/ice/documentos/redes/2012/asesoramiento/modelo-normas-apa-bibliografia.pdf
22. Lozada JS. Joint United Nations Programme on HIV/AIDS. Encycl Glob Heal 2012;
23. Althoff KN, Smit M, Reiss P, Justice AC. HIV and Ageing: Improving Quantity and Quality of Life. Curr Opin HIV AIDS [Internet] 2016;11(5):527–36. Available from: https://journals.lww.com/01222929-201609000-00012
24. Zuma K, Lurie MN, Williams BG, Mkaya-Mwamburi D, Garnett GP, Sturm AW. Risk factors of sexually transmitted infections among migrant and non-migrant sexual partnerships from rural South Africa. Epidemiol Infect 2005;133(3):421–8.
25. Pineda-Peña AC, Varanda J, Sousa JD, et al. The early spread and epidemic ignition of HIV-1 in human populations. Infect Genet Evol 2016;46:219–22.
26. United Nations Joint Programme on HIV/AIDS (UNAIDS). To help end the AIDS epidemic. United Nations [Internet] 2014;40. Available from: http://www.unaids.org/sites/default/files/media_asset/90-90-90_en.pdf
27. Lamptey PR. Regular review: Reducing heterosexual transmission of HIV in poor countries. Br Med J 2002;324(7331):207–11.
28. National Institute of Fighting against AIDS. Normas De Tratamento Antirretroviral.

2015;159. Available from: https://aidsfree.usaid.gov/sites/default/files/ao_normastratamentoarv.pdf

29. Smallman-Raynor MR, Cliff AD. Civil war and the spread of AIDS in Central Africa. Epidemiol Infect 1991;107(1):69–80.
30. Popper SJ, Sarr AD, Guèye-Ndiaye A, Mboup S, Essex ME, Kanki PJ. Low Plasma Human Immunodeficiency Virus Type 2 Viral Load Is Independent of Proviral Load: Low Virus Production In Vivo. J Virol 2000;74(3):1554–7.
31. Berry N, Jaffar S, Loeff MS van der, et al. Low Level Viremia and High CD4% Predict Normal Survival in a Cohort of HIV type-2-infected Villagers. AIDS Res Hum Retroviruses 2002;18(16):1167–73.
32. Leitner T, Hahn B, Mullins J, et al. HIV Sequence Compendium 2015 Editors. Theor Biol Biophys Los Alamos Natl Lab [Internet] 2015;Available from: https://www.hiv.lanl.gov/content/sequence/HIV/COMPENDIUM/2015/sequence2015.pdf
33. Bulanda BI, Bongenya BI, Chatte A, et al. Molecular Diversity of the Human Immunodeficiency Virus Type 1 in Metropolitan Cities in Central Africa: An Update of Data. World J AIDS 2020;10(02):80–93.
34. Hemelaar J. The origin and diversity of the HIV-1 pandemic. Trends Mol Med [Internet] 2012;18(3):182–92. Available from: http://dx.doi.org/10.1016/j.molmed.2011.12.001
35. Yamaguchi J, Vallari A, McArthur C, et al. Complete Genome Sequence of CG-0018a-01 Establishes HIV-1 Subtype L. J Acquir Immune Defic Syndr 2020;83(3):319–22.
36. Hemelaar J, Elangovan R, Yun J, et al. Global and regional molecular epidemiology of HIV-1, 1990-2015: a systematic review, global survey, and trend analysis. Lancet Infcet Dis 2018;3099(18):1–13.
37. Fauci AS. Multifactorial nature of human immunodeficiency virus disease: Implications for therapy. Science (80-) 1993;262(5136):1011–8.
38. Alcântara KC De. Epidemiologia molecular do HIV-1, resistência aos antirretrovirais em gestantes e transmissão vertical no estado de goiás. 2011;
39. Escola educação. HIV-1 life cycle [Internet]. Available from: https://escolaeducacao.com.br/transcriptase-reversa/trascrip-reversa/
40. Barré-Sinoussi F, Ross AL, Delfraissy J-F. Past, present and future: 30 years of HIV research. Nat Rev Microbiol [Internet] 2013;11(12):877–83. Available from: http://www.nature.com/doifinder/10.1038/nrmicro3132

41. Hill CM, Deng H, Unutmaz D, et al. Envelope glycoproteins from human immunodeficiency virus types 1 and 2 and simian immunodeficiency virus can use human CCR5 as a coreceptor for viral entry and make direct CD4-dependent interactions with this chemokine receptor. J Virol 1997;71(9):6296–304.
42. Fanales-Belasio E, Raimondo M, Suligoi B, Buttò S. HIV virology and pathogenetic mechanisms of infection: a brief overview. Ann Ist Super Sanità 2010;46(1):5–14.
43. Wang Z, Shang H, Jiang Y. Chemokines and chemokine receptors: Accomplices for human immunodeficiency virus infection and latency. Front Immunol 2017;8(OCT):1–12.
44. Lusso P. HIV and the chemokine system: 10 years later. EMBO J 2006;25(3):447–56.
45. Dalgleish AG, Beverley PCL, Clapham PR, Crawford DH, Greaves MF, Weiss RA. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature 1984;312(5996):763–7.
46. Klatzmann D, Champagne E, Chamaret S, et al. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature 1984;312(5996):767–8.
47. Klatt EC. Pathology of HIV/AIDS. 2019.
48. Sannier G, Dubé M, Kaufmann DE. Single-Cell Technologies Applied to HIV-1 Research: Reaching Maturity. Front Microbiol 2020;11(March):1–14.
49. Moir S, Chun T-W, Fauci AS. Pathogenic Mechanisms of HIV Disease. Annu Rev Pathol Mech Dis 2011;6(1):223–48.
50. Briggs JAG, Kräusslich HG. The molecular architecture of HIV. J Mol Biol [Internet] 2011;410(4):491–500. Available from: http://dx.doi.org/10.1016/j.jmb.2011.04.021
51. Gomez C, Hope TJ. The ins and outs of HIV replication. Cell Microbiol 2005;7(5):621–6.
52. Fraser C, Lythgoe K, Leventhal GE, et al. Virulence and Pathogenesis of HIV-1 Infection: An Evolutionary Perspective. Science (80-) [Internet] 2014;343(6177):1243727–1243727. Available from: https://www.sciencemag.org/lookup/doi/10.1126/science.1243727
53. Roper WL, Holtzman D, John Iglehart GK, et al. Revised Surveillance Case Definitions for HIV Infection Among Adults, Adolescents, and Children Aged <18 Months and for HIV Infection and AIDS Among Children Aged 18 Months to <13 Years — United States, 2008. Centers Dis Control Prev [Title] MMWR [Internet] 2008;5757. Available from: www.cdc.gov/mmwr
54. Broder S. The development of antiretroviral therapy and its impact on the HIV-1/AIDS pandemic. Antiviral Res 2010;85(1):1–18.
55. WHO. Clinical Guidelines: Antiretroviral Therapy. WHO [Internet] 2016;(Second

Edition):129. Available from: http://www.who.int/hiv/pub/arv/chapter4.pdf?ua=1

56. de Mendoza C, Lozano AB, Caballero E, Cabezas T, Ramos JM, Soriano V. Antiretroviral therapy for HIV-2 infection in non-endemic regions. AIDS Rev 2020;22(1):44–56.
57. MacArthur RD, Schaecher KL. Addressing adherence challenges associated with antiretroviral therapy: Focus on noninfectious diarrhea. Am J Manag Care 2013;19(12 SUPPL.).
58. Warnke D, Barreto J, Temesgen Z. Antiretroviral drugs. J Clin Pharmacol 2007;47(12):1570–9.
59. Henrich TJ, Kuritzkes DR. HIV-1 entry inhibitors: Recent development and clinical use. Curr Opin Virol [Internet] 2013;3(1):51–7. Available from: http://dx.doi.org/10.1016/j.coviro.2012.12.002
60. Haqqani AA, Tilton JC. Entry inhibitors and their use in the treatment of HIV-1 infection. Antiviral Res [Internet] 2013;98(2):158–70. Available from: http://dx.doi.org/10.1016/j.antiviral.2013.03.017
61. Cihlar T, Ray AS. Nucleoside and nucleotide HIV reverse transcriptase inhibitors: 25 years after zidovudine. Antiviral Res 2010;85(1):39–58.
62. Sarafianos SG, Marchand B, Das K, et al. Structure and Function of HIV-1 Reverse Transcriptase: Molecular Mechanisms of Polymerization and Inhibition. J Mol Biol [Internet] 2009;385(3):693–713. Available from: http://dx.doi.org/10.1016/j.jmb.2008.10.071
63. Anderson PL, Kakuda TN, Lichtenstein KA. The Cellular Pharmacology of Nucleoside- and Nucleotide-Analogue Reverse-Transcriptase Inhibitors and Its Relationship to Clinical Toxicities. Clin Infect Dis 2004;38(5):743–53.
64. Katherine L. Seley-Radtke, Yates MK. The evolution of nucleoside analogue antivirals: A review for chemists and non-chemists. Part 1: Early structural modifications to the nucleoside scaffold. HHS Public Access 2018;154(1):66–86.
65. Yates MK, Seley-Radtke KL. The evolution of antiviral nucleoside analogues: A review for chemists and non-chemists. Part II: Complex modifications to the nucleoside scaffold. Antiviral Res 2019;162(January):5–21.
66. Béthune MP de. Non-nucleoside reverse transcriptase inhibitors (NNRTIs), their discovery, development, and use in the treatment of HIV-1 infection: A review of the last 20 years (1989-2009). Antiviral Res 2010;85(1):75–90.
67. Flexner C. HIV-protease inhibitors. N Engl J Med 1998;338(18):1281–92.

68. Wensing AMJ, van Maarseveen NM, Nijhuis M. Fifteen years of HIV Protease Inhibitors: raising the barrier to resistance. Antiviral Res 2010;85(1):59–74.
69. Brooks KM, Sherman EM, Egelund EF, et al. Integrase Inhibitors: After 10 Years of Experience, Is the Best Yet to Come? Pharmacotherapy 2019;39(5):576–98.
70. Inzaule SC, Hamers RL, Doherty M, Shafer RW, Bertagnolio S, Rinke de Wit TF. Curbing the rise of HIV drug resistance in low-income and middle-income countries: the role of dolutegravir-containing regimens. Lancet Infect Dis [Internet] 2019;19(7):e246–52. Available from: http://dx.doi.org/10.1016/S1473-3099(18)30710-2
71. Mitsuya H, Weinhold KJ, Furman PA, et al. 3'-Azido-3'-deoxythymidine (BW A509U): An antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus in vitro. Proc Natl Acad Sci U S A 1985;82(20):7096–100.
72. Bayu B, Tariku A, Bulti AB, Habitu YA, Derso T, Teshome DF. Determinants of virological failure among patients on highly active antiretroviral therapy in University of Gondar Referral Hospital, Northwest Ethiopia: A case-control study. HIV/AIDS - Res Palliat Care 2017;9:153–9.
73. Margolis AM, Heverling H, Pham PA, Stolbach A. A Review of the Toxicity of HIV Medications. J Med Toxicol 2014;10(1):26–39.
74. Jochmans D. Novel HIV-1 reverse transcriptase inhibitors. Virus Res 2008;134(1–2):171–85.
75. Programme A. World health organization global strategy for the surveillance and monitoring of hiv drug resistance 2012. 2012;
76. Hiebl J, Zbiral E, Balzarini J, Clercq E De. Side-Chain Derivatives of Biologically Active Nucleosides. 1. Side-Chain Analogs of 3'-Azido-3'-deoxythymidine (AZT). Am Chem Soc 1992;35:3016–23.
77. Roy V, Obikhod A, Zhang H-W, et al. Synthesis and Anti-HIV Evaluation of 3'- Triazolo Nucleosides. Nucleosides, Nucleotides and Nucleic Acids 2011;(September):37–41.
78. Lazrek HB, Taourirte M, Oulih T, et al. Synthesis and anti-HIV activity of new modified 1,2,3-triazole acyclonucleosides. Nucleosides, Nucleotides and Nucleic Acids 2001;20(12):1949–60.
79. Hirota K, Hosono H, Kitade Y, et al. Synthesis and Anti-Human Immunodeficiency Virus (HIV-1) Activity of 3'-deoxy-3'-(triazol-1-yl)thymidines and 2',3'-dideoxy-3'-(triazol-1-yl)uridines and Inhibition of Reverse Transcriptase by Their 5'-triphosphates. Chem Pharm Bull

1986;34(1):430–3.

80. Wigerinck P, Aerschot A Van, Janssen G, et al. Synthesis and Antiviral Activity of 3'-Heterocyclic Substituted 3'-Deoxythymidines. J Med Chem 1990;868–73.
81. Hong-wang Z, Coats SJ, Bondada L, et al. Synthesis and evaluation of 3'-azido-2',3'-dideoxypurine nucleosides as inhibitors of human immunodeficiency virus. Bioorg Med Chem Lett [Internet] 2011;23(1):1–7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf
82. Sirivolu VR, Vernekar SK V., Ilina T, Myshakina NS, Parniak MA, Wang Z. Clicking 3'-Azidothymidine into Novel Potent Inhibitors of Human Immunodeficiency Virus. J Med Chem 2013;1(56):8765–80.
83. Kolb HC, Finn MG, Sharpless KB. Click Chemistry: Diverse Chemical Function from a Few Good Reactions. Angew Chemie - Int Ed 2001;40(11):2004–21.
84. Jiang X, Hao X, Jing L, et al. Recent applications of click chemistry in drug discovery. Expert Opin Drug Discov [Internet] 2019;14(8):779–89. Available from: https://doi.org/10.1080/17460441.2019.1614910
85. Zhan P, Pannecouque C, De Clercq E, Liu X. Anti-HIV Drug Discovery and Development: Current Innovations and Future Trends. J Med Chem 2016;59(7):2849–78.
86. Richman DD, Margolis DM, Delaney M, Greene WC, Hazuda D, Pomerantz RJ. The Challenge of Finding a Cure for HIV Infection. Science (80-) 2009;323(March):1304–7.
87. Rosemary A, Chika O, Jonathan O, et al. Genotyping performance evaluation of commercially available HIV-1 drug resistance test. PLoS One 2018;13(6):1–10.
88. Ndashimye E, Arts EJ. The urgent need for more potent antiretroviral therapy in low-income countries to achieve UNAIDS 90-90-90 and complete eradication of AIDS by 2030. Infect Dis Poverty 2019;8(1):1–8.
89. Rhee SY, Kassaye SG, Barrow G, Sundaramurthi JC, Jordan MR, Shafer RW. HIV-1 transmitted drug resistance surveillance: shifting trends in study design and prevalence estimates. J Int AIDS Soc 2020;23(9):1–12.
90. Yu F, Wen Y, Wang J, et al. The Transmission and Evolution of HIV-1 Quasispecies within One Couple: A Follow-up Study based on Next-Generation Sequencing. Sci Rep [Internet] 2018;8(1):1–8. Available from: http://dx.doi.org/10.1038/s41598-018-19783-3
91. Ssemwanga D, Lihana RW, Ugoji C, et al. Update on HIV-1 acquired and transmitted drug resistance in Africa. AIDS Rev 2015;17(1):3–20.

92. Johnson VA, Calvez V, Günthard HF, et al. 2011 Update of the Drug Resistance Mutations in HIV-1. Top Antivir Med 2011;156–64.
93. Wang Y, Xing H, Liao L, et al. The development of drug resistance mutations K103N Y181C and G190A in long term Nevirapine-containing antiviral therapy. AIDS Res Ther 2014;11(1):1–9.
94. UNAIDS. 90-90-90: An ambitious treatment target to help end the AIDS epidemic. United Nations [Internet] 2014;40. Available from: http://www.unaids.org/sites/default/files/media_asset/90-90-90_en.pdf
95. United Nations. Political Declaration on HIV and AIDS: On the Fast Track to Accelerating the Fight against HIV and to Ending the AIDS Epidemic by 2030. 2016;17020(June):1–26. Available from: https://www.unaids.org/sites/default/files/media_asset/2016-political-declaration-HIV-AIDS_en.pdf
96. Fauci AS, Redfield RR, Sigounas G, Weahkee MD, Giroir BP. Ending the HIV Epidemic: A Plan for the United States. JAMA 2019;
97. Perno C-F, Moyle G, Tsoukas C, Ratanasuwan W, Gatell J, Schechter M. Overcoming Resistance to Existing Therapies in HIV-infected Patients: The Role of New Antiretroviral Drugs. J Med Virol 2008;80:565–76.
98. S.M. H, J.J. EJ, P. R, et al. Antiretroviral treatment of adult HIV infection: 2008 Recommendations of the international AIDS society-USA panel. JAMA - J Am Med Assoc [Internet] 2008;300(5):555–70. Available from: http://jama.ama-assn.org/cgi/reprint/300/5/555%5Cnhttp://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed8&NEWS=N&AN=2008378344
99. World Health Organization, United Nations Programme on HIV/AIDS (UNAIDS). HIV in pregnancy: Review. WHO 1998;
100. MSAC. Genotypic resistance testing of antiretrovirals in HIV. 2009.
101. National Institute of Statistics. Inquérito de Indicadores Múltiplos e de Saúde (IIMS) em Angola. [Internet]. 2016. Available from: https://dhsprogram.com/pubs/pdf/FR327/FR327.pdf
102. Hogben M, Leichliter JS. Social Determinants and Sexually Transmitted Disease Disparities. Sex Transm Dis 2008;35(Supplement):S13–8.
103. National Institute of Fighting against AIDS. Plano Estratégico Nacional Para o Controlo das Infecções de Transmissão Sexual , VIH e SIDA Instituto Nacional de Luta Contra a Sida.

2006;Available from: http://www.nationalplanningcycles.org/sites/default/files/country_docs/Angola/hiv_plan_angola.pdf

104. Hirsch MS, D'Aquila RT. Therapy for human immunodeficiency virus infection. N Engl J Med [Internet] 1993;328(23):1686–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8387640
105. Clemente S. Epidemiologia molecular da infecção por VIH/SIDA, em Angola. Univ Lisboa, Fac Med Lisboa 2008;
106. Abecasis A, Paraskevis D, Epalanga M, Fonseca M, Burity F. HIV-1 genetic variants circulation in the North of Angola. Infect Genet Evol 2005;5:231–7.
107. Bártolo I, Epalanga M, Bartolomeu J, et al. High Genetic Diversity of Human Immunodeficiency Virus Type 1 in Angola. AIDS Res Hum Retroviruses 2005;21(4):306–10.
108. Bártolo I, Rocha C, Bartolomeu J, et al. Highly divergent subtypes and new recombinant forms prevail in the HIV/AIDS epidemic in Angola: New insights into the origins of the AIDS pandemic. Infect Genet Evol 2009;9(4):672–82.
109. Pineda-Peña A-C, Varanda J, Sousa JD de, et al. On the contribution of Angola to the initial spread of HIV-1. Infect Genet Evol 2016;46:219–22.
110. Afonso JM, Bello G, Guimarães ML, Sojka M, Morgado MG. HIV-1 Genetic Diversity and Transmitted Drug Resistance Mutations among Patients from the North, Central and South Regions of Angola. PLoS One 2012;7(8).
111. Bártolo I, Zakovic S, Martin F, et al. HIV-1 diversity, transmission dynamics and primary drug resistance in Angola. PLoS One 2014;9(12):1–17.
112. Castelbranco EPAF, da Silva Souza E, Cavalcanti AMS, Martins AN, de Alencar LCA, Tanuri A. Frequency of Primary Resistance to Antiretroviral Drugs and Genetic Variability of HIV-1 Among Infected Pregnant Women Recently Diagnosed in Luanda-Angola. AIDS Res Hum Retroviruses [Internet] 2010;26(12):1313–6. Available from: http://www.liebertonline.com/doi/abs/10.1089/aid.2010.0111
113. Garrido C, Zahonero N, Fernándes D, et al. Subtype variability, virological response and drug resistance assessed on dried blood spots collected from HIV patients on antiretroviral therapy in Angola. J Antimicrob Chemother 2008;61(3):694–8.
114. Lai A, Ciccozzi M, Franzetti M, et al. Local and Global Spatio-Temporal Dynamics of HIV-1 Subtype F1. J Med Virol 2014;86:186–92.

115. Bello G, Afonso JM, Morgado MG. Phylodynamics of HIV-1 subtype F1 in Angola, Brazil and Romania. Infect Genet Evol [Internet] 2012;12(5):1079–86. Available from: http://dx.doi.org/10.1016/j.meegid.2012.03.014
116. Hemelaar J. Implications of HIV diversity for the HIV-1 pandemic. J Infect [Internet] 2013;66(5):391–400. Available from: http://dx.doi.org/10.1016/j.jinf.2012.10.026
117. Sebastião CS, Morais J, Brito M. Factors Influencing HIV Drug Resistance among Pregnant Women in Luanda, Angola: Findings from a Cross-Sectional Study. Trop Med Infect Dis 2021;6(1):29.
118. Kassa D, Gebremichael G, Tilahun T, et al. Prevalence of sexually transmitted infections (HIV, hepatitis B virus, herpes simplex virus type 2, and syphilis) in pregnant women in Ethiopia: Trends over 10 years (2005–2014). Int J Infect Dis 2019;79:50–7.
119. Manyahi J, Jullu BS, Abuya MI, et al. Prevalence of HIV and syphilis infections among pregnant women attending antenatal clinics in Tanzania, 2011 Disease epidemiology - Infectious. BMC Public Health 2015;15(1):1–9.
120. Dionne-Odom J, Mbah R, Rembert NJ, et al. Hepatitis B, HIV, and Syphilis Seroprevalence in Pregnant Women and Blood Donors in Cameroon. Infect Dis Obstet Gynecol 2016;2016:1–8.
121. Gupta S, Gupta R, Singh S. Seroprevalence of HIV in pregnant women in North India: A tertiary care hospital based study. BMC Infect Dis 2007;7:4–8.
122. Costa ZB, Machado GC, Avelino MM, et al. Prevalence and risk factors for Hepatitis C and HIV-1 infections among pregnant women in Central Brazil. BMC Infect Dis 2009;9:1–9.
123. Hill SC, Neto de Vasconcelos J, Granja BG, et al. Early Genomic Detection of Cosmopolitan Genotype of Dengue Virus Serotype 2, Angola, 2018. Emerg Infect Dis [Internet] 2019;25(4):784–7. Available from: http://wwwnc.cdc.gov/eid/article/25/4/18-0958_article.htm
124. Ministry of Health of Angola. Ongoing Dengue Epidemic — Angola, June 2013. MMWR 2013;62(24):504–7.
125. Stockdale AJ, Geretti AM. Chronic hepatitis B infection in sub-Saharan Africa: A grave challenge and a great hope. Trans R Soc Trop Med Hyg 2015;109(7):421–2.
126. Etame Sone, PhD LH, Voufo, MSc RA, Dimodi, PhD HT, et al. Prevalence and Identification of Serum Markers Associated with Vertical Transmission of Hepatitis B in Pregnant Women in Yaounde, Cameroon. Int J Matern Child Heal AIDS 2017;6(1):69–74.
127. Opaleye OO, Igboama MC, Ojo JA, Odewale G. Seroprevalence of HIV, HBV, HCV, and HTLV

among Pregnant Women in Southwestern Nigeria. J Immunoass Immunochem 2016;37(1):29–42.

128. Mbangiwa T, Kasvosve I, Anderson M, et al. Chronic and occult hepatitis B virus infection in pregnant women in Botswana. Genes (Basel) 2018;9(5):1–13.
129. Mutagoma M, Balisanga H, Sebuhoro D, et al. Hepatitis C virus and HIV co-infection among pregnant women in Rwanda. BMC Infect Dis 2017;17(1):4–9.
130. Elsheikh RM, Daak AA, Elsheikh MA, Karsany MS, Adam I. Hepatitis B virus and hepatitis C virus in pregnant Sudanese women. Virol J 2007;4:8–10.
131. Frempong MT, Ntiamoah P, Annani-Akollor ME, et al. Hepatitis B and C infections in HIV-1 and non-HIV infected pregnant women in the Brong-Ahafo Region, Ghana. PLoS One 2019;14(7):1–13.
132. Loarec A, Carnimeo V, Molfino L, et al. Extremely low hepatitis C prevalence among HIV co-infected individuals in four countries in sub-Saharan Africa. Aids 2019;33(2):353–5.
133. Taremwa IM, Twelwanike A, Mwambi B, Atuhairwe C. Laboratory assessment of SD Bioline HIV/Syphilis Duo Kit among pregnant women attending antenatal clinic Mayuge Health Center III, East central Uganda. BMC Res Notes 2019;12(1):238.
134. Lawi JDT, Mirambo MM, Magoma M, et al. Sero-conversion rate of Syphilis and HIV among pregnant women attending antenatal clinic in Tanzania: A need for re-screening at delivery. BMC Pregnancy Childbirth 2015;15(1):1–7.
135. WHO. Dual HIV/Syphilis Rapid Diagnostic Tests Can Be Used As the First Test in Antenatal Care [Internet]. 2019. Available from: https://www.who.int/
136. INE M. Inquérito de Indicadores Múltiplos e de Saúde 2015-2016(IIMS). 2017;1–559.
137. National Institute of Statistics. Resultados definitivos do recenseamento geral da população e da habitação de Angola - 2014 [Internet]. 2016. Available from: http://www.effaangola.org/AngolaCensus2014_ResultadosDefinitivos_Mar2016.pdf
138. Stoddard ST, Forshey BM, Morrison AC, et al. House-to-house human movement drives dengue virus transmission. Proc Natl Acad Sci U S A 2013;110(3):994–9.
139. Guzman MG, Harris E. Dengue. Lancet 2015;385(9966):453–65.
140. Hill SC, Vasconcelos J, Neto Z, et al. Emergence of the Zika virus Asian lineage in Angola: an outbreak investigation. Lancet Infect Dis [Internet] 2019;19(10):1138–47. Available from: http://dx.doi.org/10.1016/S1473-3099(19)30293-2
141. Afonso JM, Morgado MG, Bello G. Evidence of multiple introductions of HIV-1 subtype C in

Angola. Infect Genet Evol [Internet] 2012;12(7):1458–65. Available from: http://dx.doi.org/10.1016/j.meegid.2012.05.005

142. Wallis CL, Godfrey C, Fitzgibbon JE, Mellors JW. Key Factors Influencing the Emergence of Human Immunodeficiency Virus Drug Resistance in Low- and Middle-Income Countries. J Infect Dis 2017;216(Suppl 9):851–6.
143. Van de Vijver D, Wensing AM, Boucher C, others. The epidemiology of transmission of drug resistant HIV-1. Reviews 2006;2007:17–36.
144. Perrin L, Kaiser L, Yerly S. Travel and the spread of HIV-1 genetic variants. Lancet Infect Dis 2003;3(January):22–7.
145. WHO. HIV Drug Resistance Report 2019 [Internet]. 2019. Available from: http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Who+hiv+drug+resistance+report+2012#5
146. Alencar D, Gonçalves J, Cerqueira SA, Soares H, Petronilho A. Development of Triazoles based on AZT and their Anti-Viral Activity Against HIV-1. bioRxiv 2019;
147. Stein DS, Moore KHP. Phosphorylation of nucleoside analog antiretrovirals: A review for clinicians. Pharmacotherapy 2001;21(1):11–34.

ANEXOS

MEDICAL SCHOOL
FACULDADE DE CIÊNCIAS MÉDICAS

UNIVERSIDADE NOVA DE LISBOA

Exmo. Senhor
Dr. Cruz dos Santos Sebastião
Instituto Nacional de Investigação Agrária Veterinária
IP, Avenida da Répubica
2784-505 OEIRAS

SUA REFERÊNCIA	SUA COMUNICAÇÃO DE	NOSSA REFERÊNCIA	DATA
		0324	25 MAR 2019

ASSUNTO: **INTENÇÃO DE DOUTORAMENTO EM BIOMEDICINA (3.ª EDIÇÃO): DR. CRUZ DOS SANTOS SEBASTIÃO.**

Comunico a V. Exa. que o Presidente do Conselho Científico da Faculdade de Ciências Médicas | NOVA Medical School da Universidade NOVA de Lisboa (FCM|NMS/UNL), por delegação de poderes do plenário, deliberou em 20 de março 2019:

"O Conselho Científico aprova a Intenção de Doutoramento em Biomedicina, tendo em conta o parecer da Coordenadora do Ciclo de Estudos".

Com os melhores cumprimentos,

Sónia Tavares

Dra. Sónia Tavares
Coordenadora da Secção de Pós-Graduação da Divisão Académica

ST/cp

www.nms.unl.pt

Decisão final sobre o projecto " To identify the primary resistance mutations in HIV-1 infected pregnant women associated with vertical transmission at the Lucrecia Paim Maternity during the second quarter of 2018"

A Comissão de Ética da NMS|FCM-UNL (CEFCM) decidiu, por unanimidade, aprovar, do ponto de vista ético, o projecto de investigação intitulado " *To identify the primary resistance mutations in HIV-1 infected pregnant women associated with vertical transmission at the Lucrecia Paim Maternity during the second quarter of 2018*" (nº51/2019/CEFCM), submetido por Dr. Rui Miguel Duque de Brito.

Lisboa, 29 de Julho de 2019

O Presidente da Comissão de Ética,

(Prof. Doutor Diogo Pais)

TO WHOM IT MAY CONCERN

The Ethics Research Committee NMS|FCM-UNL (CEFCM) has unanimously, approved the Project entitled " *To identify the primary resistance mutations in HIV-1 infected pregnant women associated with vertical transmission at the Lucrecia Paim Maternity during the second quarter of 2018*" (nr.51/2019/CEFCM), submitted by Rui Miguel Duque de Brito MD.

Lisbon, July 29th, 2019

The Chairman of the Ethics Research Committee,

(Diogo Pais, MD, PhD)

REPÚBLICA DE ANGOLA
MINISTÉRIO DA SAÚDE

COMITÉ DE ÉTICA

Nº 13 2018

Parecer sobre o projecto de pesquisa intitulado «**Mutações de Resistência Primária em Gestantes Infectadas Pelo VIH Associado a Transmissão Vertical na Maternidade Lucrécia Paím Durante o Segundo Trimestre de 2018**», submetido a este Comité pelo **Dr. Cruz dos Santos Sebastião,** Doutorando em Biomedicina na Universidade de Ciências Médicas da Universidade Nova Lisboa.

A leitura e análise da proposta do protocolo em epígrafe, permitiu ao Comité de Ética constatar que, o parecer é «**Positivo**» porque o seu estudo enquadra-se na implementação de um programa de prevenção e controlo de infecções e pode contribuir na definição de uma terapia direcionada capaz de melhorar a qualidade de vida das pacientes e reduzir a transmissão de cepas resistente, uma vez que o estudo visa avaliar as mutações de resistência primária em gestantes infectadas pelo VIH associado a transmissão vertical na maternidade Lucrécia Paím durante o segundo trimestre de 2018.

Contudo, deve afirmar-se que qualquer possibilidade ou vontade de publicação de dados advindos do estudo, deve ser primeiro e solicitado ao Ministério da Saúde bem como ao Comité de Ética do mesmo.

LUANDA, AOS 26 DE MARÇO DE 2018.

A PRESIDENTE DO C.E

DRA. JOANA FILIPA M. M. AFONSO

REPÚBLICA DE ANGOLA
MINISTÉRIO DA SAÚDE
MATERNIDADE LUCRÉCIA PAIM
GABINETE DA DIRECTORA GERAL

Ao

Instituto Nacional de Saúde Pública

Gabinete da Directora Geral

Att. Drª Joana Filipa M. de M. Afonso

= LUANDA=

OFICIO Nº. 083/GDG/MLP/2018.

Os Nossos Melhores Cumprimentos.

Em conformidade com a vossa solicitação datado de 20 de Março do corrente ano, na qual solicitam a autorização de recolha de dados para o senhor **Cruz dos Santos Sebastião** na nossa instituição, sob o tema **Mutações de resistência primária em gestantes infectadas pelo VIH associado a transmissão vertical na Maternidade Lucrécia Paim durante o segundo trimestre de 2018**, apraz-nos informar que está autorizada, devendo para efeito contactar a Direcção Clínica, Direcção Cientifica Pedagógica e Chefe do Laboratório para acompanhamento.

- Solicitamos que nos seja entregue um exemplar do relatório de pesquisa.

Sem outro assunto de momento, aceite os protestos da nossa elevada estima e consideração.

GABINETE DA DIRECTORA GERAL DA MATERNIDADE LUCRÉCIA PAIM, em Luanda ao 11 de Abril de 2018.

A DIRECTORA GERAL

Adelaide de Carvalho

ADELAIDE DE CARVALHO

Endereço: Luanda na Avenida Comandante Gika, Largo Sagrada Família,
Tel. 222 322395, email: mlpinforme@gmail.com

MEDICAL SCHOOL
FACULDADE DE CIÊNCIAS MÉDICAS

UNIVERSIDADE NOVA DE LISBOA

Exmo. Senhor
Dr. Cruz dos Santos Sebastião
Instituto Nacional de Investigação Agrária
2784-505 OEIRAS

SUA REFERÊNCIA	SUA COMUNICAÇÃO DE	NOSSA REFERÊNCIA	DATA
		0072	16 JAN 2020

ASSUNTO: **ALTERAÇÃO DO TÍTULO DA TESE DE DOUTORAMENTO EM BIOMEDICINA (3.ª EDIÇÃO): DR. CRUZ DOS SANTOS SEBASTIÃO.**

Comunico a V. Exa. que o Presidente do Conselho Científico da Faculdade de Ciências Médicas | NOVA Medical School da Universidade NOVA de Lisboa (FCM|NMS/UNL), por delegação de poderes do plenário, deliberou, em 10 de janeiro de 2020, autorizar o pedido de alteração do título da Tese de Doutoramento em Biomedicina.

Neste âmbito, e conforme solicitado no seu requerimento de 2/12/2019, a tese de Doutoramento em Biomedicina intitulada ***""Mutações de resistência primária em gestantes infectadas pelo VIH associado a transmissão vertical na Maternidade Lucrécia Paim durante o segundo trimestre de 2018."*** passa a ter o novo título ***"HIV-1 infection among pregnant women in Angola: Molecular epidemiology and test of novel AZT triazole derivatives"***.

Com os melhores cumprimentos,

Patrícia Paiva

Dra. Patrícia Paiva
Divisão Académica - Secção de Pós-Graduação

PP/phd

CAMPO DOS MÁRTIRES DA PÁTRIA 130 1150-056 LISBOA PORTUGAL T. +351 218 803 000 F. +351 218 851 020 www.nms.unl.pt

Declaração

A Comissão de Ética da NMS|FCM-UNL (CEFCM) decidiu, por unanimidade, aprovar a alteração ao titulo do projeto de investigação intitulado "To identify the primary resistance mutations in HIV-1 infected pregnant women associated with vertical transmission at the Lucrecia Paim Maternity during the second quarter of 2018" para "***HIV-1 infection among pregnant women in Angola: Molecular epidemiology and test of novel AZT triazole derivatives***" (nº 51/2019/CEFCM), do doutorando Cruz dos Santos Sebastião, no âmbito do Doutoramento em Biomedicina, submetido pelo Prof. Doutor Rui Miguel Duque de Brito.

Lisboa, 24 de fevereiro de 2021

O Presidente da Comissão de Ética,

(Professor Doutor Diogo Pais)

APÊNDICE A – CONSENTIMENTO INFORMADO

Você está sendo convidada como voluntária a participar da pesquisa sobre mutações de resistência primária em gestantes infectadas pelo VIH associado a transmissão vertical na maternidade Lucrécia Paím durante o segundo trimestre de 2018.

O motivo que nos leva a estudar o problema é que a presença de vírus resistente aos anti-retrovirais em gestantes constitui um grande problema de saúde pública, visto que, em casos de transmissão vertical, a criança recebe já cepas resistentes que acaba dificultando o tratamento no recém-nascido.

Durante a pesquisa, faremos colheita de dados sociodemográficos, teste do VIH e em caso de resultado positivo, iremos colher amostra de sangue em papel de filtro DBS e seguir a gestação até o nascimento, em seguida iremos testar a criança para verificar a presença ou não da infecção pelo VIH e garantir melhor qualidade de vida da mãe e do recém-nascido.

Você é livre para recusar a participação, retirar seu consentimento ou interromper a participação a qualquer momento.

Eu (gestante) _____declaro ter lido e compreendido este documento, bem como as informações verbais que me foram fornecidas pelo pesquisador. Foi-me garantida a possibilidade de a qualquer altura, recusar participar neste estudo sem qualquer tipo de penalidades. Desta forma, **aceito** participar neste estudo e permito a utilização dos dados que de forma voluntária forneço, confiando em que apenas serão utilizados para esta investigação e nas garantias de confidencialidade que me foram dadas pelo investigador.

Luanda, aos _____ de _____de 2018